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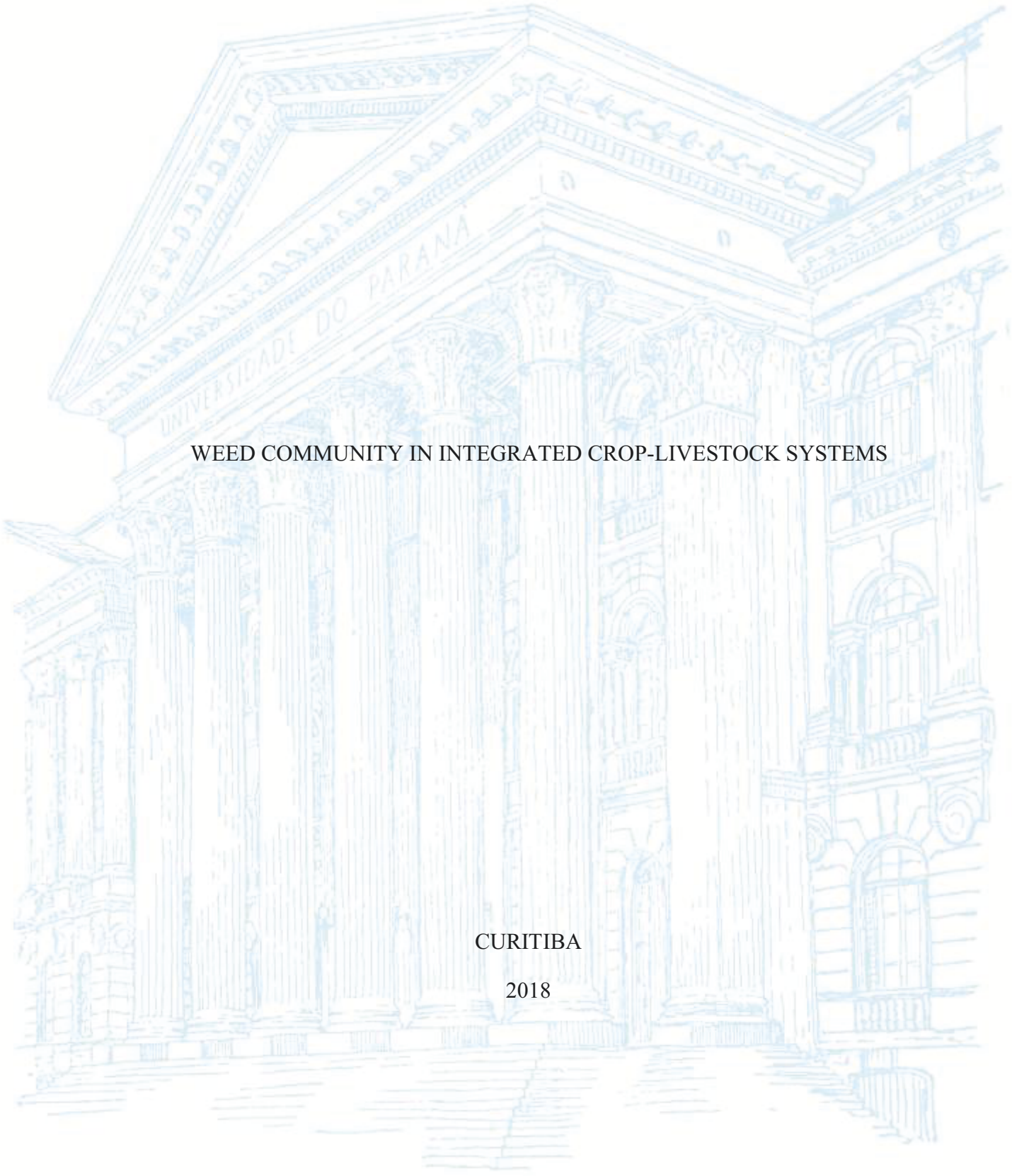

WEED COMMUNITY IN INTEGRATED CROP-LIVESTOCK SYSTEMS

CURITIBA

2018

MAURÍCIO ZANOVELLO SCHUSTER

WEED COMMUNITY IN INTEGRATED CROP-LIVESTOCK SYSTEMS

Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanitarismo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Doutor em Ciências.

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Muito obrigado!

"A smooth sea never made a skilled sailor"

(Franklin D. Roosevelt)

COMUNIDADE DE PLANTAS DANINHAS NOS SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA

RESUMO

Sistemas de produção com componentes cada vez mais diversificados e dinâmicos, como o Sistema Integrado de Produção Agropecuária (SIPA), criam a complexidade que desloca e modifica a comunidade de plantas daninhas ao longo das sucessões espaço-temporais do cultivo de pastagens e lavouras, o que, entre outras coisas, impede a seleção contínua de espécies específicas de plantas daninhas adaptadas a um tipo de cultura. Isso pode ser útil para o manejo de plantas daninhas, gerar economia com a redução do uso de herbicidas e ainda promover a biodiversidade nas propriedades agrícolas. Entretanto, a magnitude dos efeitos dos SIPA sobre a comunidade de plantas daninhas depende do manejo empregado nas pastagens e nas culturas agrícolas. Impactos dos SIPA sobre plantas daninhas foram estudados com variações de práticas de manejo em quatro abordagens: (1) Em um experimento de longa duração com integração da produção de soja no verão e bovinos de corte em pastejo no inverno foi avaliado o efeito de diferentes alturas de manejo do pasto (10, 20, 30 e 40 cm) no banco de sementes e na emergência de plantas daninhas, o qual mostrou que o aumento da altura do pasto reduziu o tamanho do banco de sementes e a emergência de plantas daninhas no inverno e no verão. (2) Um estudo de campo com rotação de culturas durante o verão de soja-milho ou soja-soja em sucessão com culturas de cobertura pastejada no inverno com diferentes manejos de pastoreio (métodos de lotação e ofertas de forragem contrastantes) permitiu identificar, ao longo destes manejos padrões dos SIPA, que a maior oferta de forragem (ou seja, aumentar a altura de manejo do pasto) foi a principal prática que proporcionou a redução da população de plantas daninhas. (3) Os levantamentos de plantas daninhas em grande escala foram realizados em vários campos comerciais, envolvendo dois protocolos experimentais (um experimento de curto prazo e um de longo prazo) no sul do Brasil e mostraram que a composição de espécies diferiu significamente de acordo com as diferentes ofertas de forragem. Diversas espécies de plantas daninhas problemáticas foram suprimidas quando foi utilizada oferta moderada de forragem (> 20 cm de altura do dossel do pasto) que também promoveu maior produção de soja e carne bovina com menor demanda de aplicação de herbicidas, quando comparados com a menor oferta de forragem (<10 cm). (4) O modelo construído com observações da dinâmica da abundância de plantas daninhas durante 12 anos na França em rotações de cereais com a inclusão de pastagem temporária (isto é, forrageira perene em rotação com milho, trigo e cevada) revelou que os efeitos da fertilização e duração da pastagem temporária na rotação podem ser mediados por variações de produção da biomassa das pastagens e das culturas. Sendo que quanto maior a quantidade de biomassa produzida menor a infestação de plantas daninhas no próximo ano.

Palavras chave: rotações de culturas e pastagens, manejo integrado de plantas daninhas, controle biológico, dinâmica da população de plantas, ecologia das plantas daninhas.

WEED COMMUNITY IN INTEGRATED CROP-LIVESTOCK SYSTEMS

ABSTRACT

Production systems that have increasingly diverse and dynamic components, such as an Integrated Crop-Livestock System (ICLS), create complexity that shifts the weed communities further along the continuum of spatial-temporal grassland-cropping succession and may be used to prevent the continuous selection of particular weed species adapted to one crop type. This might be useful for weed management, economy in herbicide applications and promoting biodiversity. However, the magnitude of the effects of ICLS on weeds depends on the management of grasslands and crops. Here, the impacts of such ICLS on weeds were studied with four approaches. (1) The first approach involved a long-term experiment that investigated the effects of different grazing intensities (10, 20, 30 and 40 cm of sward height) on weed seedling emergence and seed banks on a soybean-beef system (i.e., grazing cover crop by cattle in the winter and soybean production in the following summer). This experiment showed that an increase in pasture sward height reduced the weed seed bank and reduced weed emergence in the winter. Furthermore, it showed that an increase in pasture sward height promoted more residue of winter-grazed cover crop biomass that remained during the summer crop phases and likely resulted in a physical barrier to weed emergence. (2) The second approach involved a field experiment with contrasting crop rotation (i.e., during summer, the crop rotation treatments involved either soybean-maize or soybean-soybean in succession with winter-grazed cover crops) submitted to different grazing management options (stocking method and forage allowances) allowed identify, over the standard management of ICLS, that increased pasture sward height (higher forage allowances) was the main practices that provided weed population reduction. (3) The third approach involved conducting large-scale weed surveys in several commercial fields involving two experimental protocols (one short-term and one long-term experiment) in Southern Brazil and showed that weed species composition differed strongly among different swards heights. When compared with lower forage allowances (<10 cm sward height of winter grazed-cover crop), several problematic weed species were suppressed when using moderate forage allowances (>20 cm sward height of winter grazed-cover crop), which promoted higher soybean and beef production with reduced demands for herbicide input. (4) The fourth approach involved building a model with measurements of weed abundance dynamics on grassland-cropping rotations over 12 years in France (i.e., mowed perennial forage in rotation with maize, wheat and barley). This model revealed that the effects of the fertilization and duration of the temporary grassland included in the cereal rotation may be mediated by variations of grassland and crop biomass production and that higher biomass production reduce future weed populations.

Key-words: sod-based rotations, integrated weed management, biological control, plant population dynamics, weed ecology.

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INTRODUCTION

The world population is projected to increase 34% by 2050 (Food and Agriculture Organization [FAO], 2016), and agricultural production on existing land will have to increase to feed this population in a sustainable manner (Fedoroff et al., 2010; Robertson and Swinton, 2005). Ongoing research on integrated crop-livestock systems (ICLS) throughout the world has shown promise for improving agricultural productivity and providing synergy between agricultural production practices and environmental quality (Herrero et al., 2010; Lemaire et al., 2014).

Weeds remain a major constraint to productivity, and excessive use of herbicides has resulted in serious environmental and ecological issues. Furthermore, the increasing prevalence of herbicide-resistant weeds worldwide is concerning with respect to global food security (Busi et al., 2013). Therefore, a general call to limit the use of herbicides has occurred (Barzman and Dachbrodt-Saaydeh, 2011), and research is currently being conducted on a variety of cropping systems to develop more effective integrated weed management methods that are ultimately sustainable and reduce reliance on herbicides (Lechenet et al., 2017). However, the impacts of agronomic and livestock management practices on weed dynamics and seed bank recruitment in ICLSs are scarce and not well understood (de Moraes et al., 2014). The present study aims to reduce this gap in our knowledge with an interdisciplinary approach (agronomy and animal science) to investigate weed outcomes in ICLSs.

This thesis entitled „Weed community in integrated crop-livestock systems“ is composed of four chapters. In the first chapter, we demonstrate the long-term effects of different grazing intensities with regard to the abundance and diversity of weeds, both aboveground and in the seed bank. To accomplish this goal, we investigated both grazing and non-grazing areas. The second chapter investigated the interactions of crop rotation, forage allowance and stocking methods with respect to weed infestation in arable lands and provides recommendations of strategies for integrated weed management in an ICLS. The third chapter explores the ICLS management practices targeted towards increasing the production efficiency of the entire system. Specifically, we present evidence supporting a win-win situation for this system based on an optimum forage allowance (sward height of pasture management) that promotes higher soybean and beef production with reduced demands for herbicide input. The fourth chapter modelled the weed abundance dynamics on grassland-cropping rotations over 12 years, with the model expressing the abundance of weeds as a

function of the culture in place and the biomass produced in previous years by the grassland and crop.

This thesis concerns an area of great interest for world food security regarding major crops and cultivated pastures (i.e., soybean, maize and mixed black oat + Italian ryegrass), and our results have several potential implications for field crop ecology and management beyond the fact that forage allowance is a potential tool for biological weed control and for to reduce the herbicide dependence. The first three chapters were conducted in subtropical Southern Brazil in multiple locations and years and involved the cumulative results from farms fields, one short-term and two long-term experiments (in place for >12 years). The fourth chapter was conducted at the long-term experimental site of temporary grassland in cropping systems denominated SOERE ACBB (Observatory and Experimental System for Environmental Research - Agroecosystems, Biogeochemical Cycles, and Biodiversity; http://www.soere-acbb.com/) at the INRA Lusignan in Poitou-Charentes, France. The results are of value to the weed-management literature as well as the range landscape ecology and animal science literature. In particular, ecologists, farmers and agricultural technicians should find this thesis of value because the data clearly show how such systems can be directly used to manage weed populations in cropping systems.

CHAPTER 1

Grazing intensities affect weed seedling emergence and the seed bank in an integrated crop–livestock system[1]

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Grazing intensities affect weed seedling emergence and the seed bank in an integrated crop–livestock system

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Abstract

Integrated crop-livestock systems (ICLS) are widespread worldwide. Although weeds can result in several types of losses, essential information regarding weed seedling emergence and seed banks is lacking in these systems for integrated weed management planning. This study investigated the effects of different grazing intensities (no grazing and grazed swards maintained at 10-, 20-, 30- and 40-cm sward heights) on weed seedling emergence and seed banks in a winter cover crop during the 15th year of an ICLS experiment under no-tillage management in subtropical Southern Brazil. We hypothesized that low grazing intensities would reduce weed interference and weed seed banks size in an ICLS. We determined treatment effects on weed species richness, seed bank population density, and seedling emergence during winter and summer. Higher sward heights in the winter-grazed cover crop reduced the number of weed species, the density of emerged weed seedlings, and the weed seed bank size compared with the non-grazed control. With a sward grazing height of 10 cm, the seed bank contained an average of 3,151 seeds m^{-2}, and the weed population densities during the summer and winter were 11.8 and 21.7 plants m^{-2} greater, respectively, compared with the sward grazing height of 40 cm. Fifteen years after adopting low grazing intensities (30- and 40-cm sward heights) in the ICLS, the size of the weed seed bank was reduced by 42.1% compared with the non-grazed treatment. Decreasing the grazing intensity reduced the number of weed species, the density of emerged weed seedlings, and the weed seed bank density; therefore, integrated weed management strategies should consider minimizing grazing intensities in an ICLS.

Keywords: Ecological filters, integrated weed management, biological control, glyphosate-resistance, plant population dynamics, weed ecology

1. Introduction

Weeds reduce the potential yields of row crops and pastures, and result in an estimated annual loss of US$ 99.2 billion per year in the US, UK, Australia, India, South Africa and Brazil combined (Pimentel et al., 2001). In addition, the increased number of glyphosate-resistant weed species is a primary factor that threatens food security in global agriculture (Busi et al., 2013) and has increased the cost of controlling weeds on farms (Beckie, 2011).

Integrated crop-livestock systems (ICLS) are widespread throughout the world, comprising a total area of approximately 2.5 billion hectares (Keulen and Shiere, 2004). Studies of ICLS have been conducted with regard to production responses (Carvalho et al., 2014), environmental quality (Lemaire et al., 2013), economic viability (Oliveira et al. 2013) and social benefits (Franzluebbers et al., 2014). However, Moraes et al. (2014) published a review of 450 ICLS papers published in 93 journals between 1994 and 2013 and found that although 62% of those papers considered crop components, and only 4% considered weeds, demonstrating a gap in knowledge regarding weed management in ICLS.

Ecology-based integrated weed management in agroecosystems for modern range production depends on the ability to predict the consequences of management activities while understanding the patterns and processes of vegetation (Robertson and Swinton, 2005) and seed bank changes (Davis et al., 2006). Grazing by domestic animals is an important driver of global vegetation change (Díaz et al., 2007), and managing grazing intensity is important for understanding the overall dynamics of any ICLS (Kunrath et al., 2014).

The current work investigated the effects of different grazing intensities on weed seedling emergence and seed banks in an ICLS. We hypothesized that reduced grazing intensities would lower weed interference and weed seed banks in an ICLS. We sought to determine whether weed species composition, seedling emergence, and seed bank size change with changing grazing intensity in an ICLS; therefore, we compared the effects of

different grazing intensities on those factors during the 15th year of an ICLS experiment under no-tillage management in subtropical Southern Brazil.

2. Materials and methods

2.1 Site and treatment description

The long-term ICLS experiment used for this study was located on a 22-ha field at the Espinilho Farm (Agropecuaria Cerro Coroado) in São Miguel das Missões in Rio Grande do Sul State, Brazil (28º56'12" S latitude, 54º20'52" W longitude, 465m altitude). This site has a warm humid summer (Cfa) climate according to the Köeppen classification system, with an average temperature of 19ºC and a yearly average precipitation of 1,850 mm. The soil is a clayey Oxisol (Rhodic Hapludox; Soil Survey Staff, 1999) that is deep, well-drained and dark red with a clayey texture (540, 270 and 190 g kg^{-1}clay, silt and sand, respectively).

Before 1993, gallery forest and natural pasture covered the experimental area. The area was converted into no-tillage cropland in 1993, and from 1993 to 2001 soybeans (*Glycine max* (L.) Merr.) were grown during the summer, and black oats (*Avena strigosa* Schreb.) were grown during the winter. Since 2001, a soybean–beef cattle ICLS experiment was established with two seasons: (1) the winter season with cattle grazing a cover crop from May to November; and (2) the summer season with soybean crops from December to May.

During the winter season, black oat (cv. "Iapar 61") was sown (45 kg ha^{-1}) with naturally reseeding Italian ryegrass (*Lolium multiflorum* Lam. cv."common") to form a mixed winter pasture system; the ryegrass heads out and drops seed at the end of each winter grazing cycle and establishes itself each year by self-seeding, which is the common practice in ICLS in the region (Neto et al., 2014). Grazing was forage-based in a continuous stocking system with steers weighing approximately 200 kg (crossbred Angus, Hereford and Nellore)

that entered the pasture system when the aboveground forage mass was approximately 1.5 ton ha^{-1} of dry matter (approximately 25 cm sward height).

The treatments consisted of different grazing intensities during the winter season that were determined by pasture vegetation sward height. Sward heights of 10-, 20-, 30- and 40-cm (G10, G20, G30 and G40, respectively) were considered in addition to a reference non-grazed (NG) treatment. All treatments were organized in a randomized complete block design with three replications, with experimental units ranging from 0.8 to 3.2 ha. Sward heights for each treatment, which corresponded to grazing intensity (stocking), were controlled with variable stocking with put-and-take steers that were added or removed from the plot as required. The experimental unit size varied across treatments to achieve the desired sward height with the desired minimum number of three test animals. Sward heights were measured every 14 days using the sward stick method, using a graduated measurement stick with a sliding marker. The sward height recorded was the height at which the first forage leaf blade contacted as the marker as it was lowered into the canopy. Approximately 100 readings (points) were taken randomly in each experimental unit. The grazing period was approximately 110 days; for more details see Kunrath et al. (2014).

At the end of the winter season (mid-November), the pasture was desiccated with glyphosate (1750 g a.i. ha^{-1}), chlorimuron-ethyl (37 g a.i. ha^{-1}, used for the first 11 years) and saflufenacil (35 g a.i ha^{-1}, used for the past 4 years). The early desiccation (2-4 week before sowing) was necessary for residue management and facilitation of no-tillage soybean seeding (Bolliger et al., 2006). In mid-December of each year, soybeans were sown with the cultivar "Iguaçu" for the first three years and with "Nidera RR" (a transgenic glyphosate-resistant cultivar) in the remaining years. Soybeans were sown in rows spaced 45cm a part at a seeding rate of 45 seeds m^{-2}. Post-emergent herbicide was applied in mid-January (chlorimuron-ethyl 37 g a.i. ha^{-1} and clethodim 100 g a.i. ha^{-1} in the first three years and glyphosate at 1,400 g

a.i.ha^{-1}in the remaining years), and insecticides and fungicides were applied following agronomic recommendations. Soybeans were harvested in May each year.

2.2 Seed bank sampling and seed tray maintenance

Soil samples were collected from the central area of each plot, where 56 soil cores were taken from the top 5-cm layer.

Seed banks were sampled before summer crop seeding in November 2014, which marked the beginning of year 15 of the ICLS experiment described above. Soil samples were collected manually along four 56-m transects in each experimental unit using a steel 4.2-cm diameter probe, and 56 soil cores were extracted from the top 5 cm of the central area of each plot. The four transects were laid out in the "XX" pattern described by Wiles and Schweizer (2002) to ensure an adequate spatial distribution of weed seed bank sampling. Along the transects, two soil cores were collected at 8-m intervals and combined into one 56-core composite sample for each experimental unit.

All soil samples were processed to remove stones and root fragments, then spread in 44x38-cm plastic trays and placed in a greenhouse for 12 months beginning in November 2014. Soil moisture was maintained in the trays using regular sub-irrigation. The seedling emergence method (Thompson et al., 1997) was used to quantify the readily germinable seeds (not taking into account dead or dormant seeds) in the soil seed bank (Ma et al., 2012). During the seed tray maintenance, the lowest temperature was 0ºC, and the maximum temperature was 38ºC.

Emerged seedlings were periodically identified, counted and removed from the plastic trays. Seedling identification was conducted as described by Kissmann and Groth (1997) and Lorenzi (2006). A two-week drought period was imposed in May 2015 to break seed dormancy (De Cauwer et al., 2010). At the end of the drought period, the soil in the trays was

stirred and sub-irrigation was reactivated. After the seedling emergence ceased, the samples were stirred and placed in a room at 4ºC for three weeks before being subjected to alternating temperatures of 15 and 4ºC for one-week intervals and then returned to the greenhouse (Cardina et al., 2002). This process was repeated until no additional seedlings emerged.

2.3 Field weed seedling sampling

In each experimental unit in the field, weed seedling emergence was quantified into two seasons: at the end of grazing (November in 2014 and 2015) and during soybean cropping before post-emergent herbicide application (mid-January in 2015 and 2016). The emerged weed seedlings were identified and counted within 50x50 cm quadrats placed at 14-m intervals in the central area of each experimental unit and distributed along four 56-m transects laid out in the "XX" pattern described previously. We calculated the population density of each species from the individual number of each species per m^2. Plants were identified according to Kissmann and Groth (1997) and Lorenzi (2006).

2.4 Data analysis

Shannon's diversity index was estimated for each treatment as follows (Kent and Coker, 1992):

$$H = \sum_{i=1}^{S} \left(\frac{ni}{N}\right)\left(\log_2 \frac{ni}{N}\right)$$

where *N* is the total number of individuals per plot, *ni* refers to the number of individuals per species per plot and *S* describes the total number of species.

The evenness of the species in each treatment was also calculated using Shannon's diversity index as follows (Kent and Coker, 1992):

$$J = \frac{H}{\ln(s)}$$

Changes in the weed species richness, Shannon's diversity index, the evenness index and the seed bank population densities of *Brachiaria plantaginea* (Link) Hitchc., *Gnaphalium spicatum* Lam. and *Senecio brasiliensis* Less. had normal distributions and homogeneous variances according to the Shapiro-Wilk test (P<0.05) and Bartlett's test (P<0.05), respectively. These changes were tested using a one-way ANOVA, and treatment means were separated using the LSD test (P<0.05). The following data were not normally distributed and had heterogeneous variances according to the Shapiro-Wilk test (P<0.05) and Bartlett's test (P<0.05), respectively, and were therefore analysed using the non-parametric Kruskal-Wallis test: weed seed bank population density of *Conyza bonariensis* (L.) Conquist, *Conyza canadensis* (L.) Cronquist, *Echium plantagineum* L., *Eleusine indica* L. Gaertn, *Heteranthera reniformis* Ruiz & Pav., *Ipomoea purpurea* (L.) Roth, *Nothoscordum gracile* (Aiton.) Steam., *Phyllanthus tenellus* Roxb., *Plantago tomentosa* Lam., *Richardia brasiliensis* Gomes, *Sida rhombifolia* L., *Solanum americanum* Mill., *Soliva pterosperma* (Juss.) Less., *Stachys arvensis* L., *Stellaria media* (L.) Vill., *Xanthium strumarium* L. and seed bank size; summer weed seedling density of *C. bonariensis*, *Cyperus esculentus* L., *Digitaria horizontalis* Wild., *E. indica*, *H. reniformis*, *P. tenellus*, *R. brasiliensis*, *S. brasiliensis*, *S. americanum*, *X. strumarium* and total seedling emergence; and winter weed seedling density of *C. canadensis*, *E. plantagineum*, *G. spicatum*, *H. reniformis*, *N. gracile*, *P. tenellus*, *S. brasiliensis*, *S. americanum*, and *S. arvensis.* Treatment comparisons were completed using the Mann-Whitney U test (P<0.05). Weed seedling emergence during the winter (2014 and 2015) and summer (2015 and 2016) did not significantly differ between the sampling years (P>0.80) according to a one-way ANOVA and a Mann-Whitney U test (P<0.05), regardless of whether the data were normally distributed.

The relationship between the weed community of the seed banks and seedling emergence (winter+summer) was tested using non-metric multidimensional scaling (NMDS). The dissimilarity matrix was calculated using the Bray-Curtis index (Bray and Curtis, 1957):

$$BC_{jk} = \frac{\sum_{i=1}^{s} 2a_{ij} - a_{ik}}{\sum_{i=1}^{s} a_{ij} + \sum_{i=1}^{s} a_{ik}}$$

where BC_{ij} is the dissimilarity between sites j and k, a_{ij} and a_{ik} are the relative species abundance of species i in sites j and k, respectively, and S is the combined total abundance of the species in both communities. All ordinations were based on the Bray-Curtis index for constructed NMDS following Ma et al. (2014). To test for differences in the weed community among treatments, a PERMANOVA of the dissimilarity matrix was conducted with 9,999 Monte-Carlo iterations following McKenzie et al. (2016).

3. Results

3.1 Weed seed bank size, emerged seedling density and species richness

Grazing intensity affected the weed seed bank size (P<0.01) and weed emergence during the summer (P<0.01) and winter (P<0.001) seasons. Treatments with 15 years of low grazing intensity (G30 and G40) had fewer weeds emerge compared with the other grazing treatments (Figure 1). Moderate grazing intensities (G20) reduced weed emergence compared with a high grazing intensity (G10); however weed emergence in the no grazing treatment was equivalent to the moderate grazing intensity treatment during the summer period. The G10 grazing intensity treatment resulted in an average weed seed bank size of 3,150 and in seedling population densities during the summer and winter that were 12 and 22 individuals m^{-2} higher than the G40, respectively. No emerged weed seedlings were found in the NG treatment during the winter (Figure 1).

The species richness of the weed seed bank and the number of seedlings that emerged during the summer and winter decreased significantly (P<0.0001) as sward height increased (Figure 1); however, no significant differences were observed in seed bank richness between G30 and G40. Similarly, emerged seedling species richness did not differ between G30 and G40 during the winter and did not differ among G30, G40, and NG in the summer.

3.2 Species compositions of weed seed bank and emerged seedlings

Shannon's diversity index was affected by pasture management (P<0.0001). The G10 treatment resulted in higher Shannon's diversity indices than G30, G40, or NG (Table 1). According to the LSD test (P<0.05), the winter and summer Shannon‟s indices for vegetation did not significantly differ between G10 and G20 (Table 1); however, the seed bank Shannon index for G10 was significantly higher than for the other treatments (Table 1). Treatment significantly affected species evenness (P<0.0001). Species evenness for vegetation was lower for G10 than for G30 and G40. Seed bank evenness in G10 did not differ from G20, and the G10 and G20 evenness values were significantly lower than those for G30, G40, and NG (Table 1). *G. spicatum*, *C. canadensis*, and *R. brasiliensis* dominated the soil seed bank, together accounting for 55.3% of the soil seed bank. The dominant weed seedlings were *C. canadensis* during the winter and *B. plantaginea* and *R. brasiliensis* during the summer (50.3% and 42.2% of the seedlings, respectively for winter and summer). *Rumex obtusifolius* L. and *S. media* were found only in the weed seed bank (Table 2).

The species in the weed seed bank that were significantly affected by the grazing treatments included *B. plantaginea* (P<0.0001), *C. canadensis* (P<0.05), *G. spicatum* (P<0.001), *R. brasiliensis* (P<0.001), *N. gracile* (P<0.05), *S. brasiliensis* (P<0.002), *S. rhombifolia* (P<0.05), *S. arvensis* (P<0.05) and *S. pterosperma* (P<0.05; Table 2). For *C. canadensis, G. spicatum, R. brasiliensis* and *S. brasiliensis*, treatment G10 contained more

seeds m^{-2} than all other treatments (P<0.05). The number of *B. plantaginea* seeds was greatest in the G10 treatment, intermediate in NG and G20, and least in the G30 and G40 treatments (P<0.05). For *S. rhombifolia*, the seed numbers in the NG and G10 treatments were greater than those in all other treatments. *S. arvensis* and *S. pterosperma* were found in the NG, G10 and G20 treatments, with higher seed population densities in the G10 treatment than in the NG and G20 treatments. *C. bonariensis, E. plantagineum*, *P. tenellus* and *N. gracile* were identified in the G10 and G20 treatments, but the only significant difference between the G10 and G20 treatments was observed for *N. gracile*. The *C. esculentus, Facelis retusa* (Lam.) Schultz-Bip. and *Taraxacum officinale* F.H. Wigg., which were identified only in the G10 treatment (Table 2).

The weed seedling emergence of *C. canadensis* and *S. brasiliensis* varied significantly among grazing treatments (P<0.0001 and P<0.0004, respectively) during the winter. The average population of *C. canadensis* in G10 was 10.4 individuals m^{-2} greater than the average population of *C. canadensis* in G40 (Table 2). *S. brasiliensis* was not identified in the G40 treatment, and fewer individuals of that species were observed in G30 than in the G10 and G20 treatments (Table 2). *C. bonariensis*, *D. horizontalis*, *E. indica, F. retusa*, *P. tomentosa*, *R. brasiliensis*, *S. pterosperma* and *T. officinale* were identified only in the G10 treatment. In addition, *E. plantagineum*, *P. tenellus*, *S. americanum* and *S. arvensis* were identified only in the G10 and G20 treatments, with no significant differences between the treatment (P<0.05). Furthermore, these weed seedling species were not identified in the NG treatment.

Regarding summer weed species, significant differences were observed among the numbers of *B. plantaginea* (P<0.0007), *C. canadensis* (P<0.002), *D. horizontalis* (P<0.0004), *R. brasiliensis* (P<0.0004) and *S. rhombifolia* (P<0.001). No significant differences among the NG, G10 and G20 treatments were observed for *B. plantaginea*, *D. horizontalis*, or *R. brasiliensis*; however, significant differences were observed between the other treatments

(P<0.05). *C. canadensis* and *S. rhombifolia* were not identified in the NG treatment, and the population densities of those species were greatest in the G10 treatment. *C. canadensis*, *D. horizontalis* and *R. brasiliensis* were not identified in the G40 treatment, and the highest seedling population densities of these species occurred in the G10 treatment.

3.3 Similarity between the weed seed bank and the emerged seedlings

The NMDS results (Figure 2) show that a continuous shift in the species composition of weed vegetation (emerged seedlings) occurred along the grazing intensity gradient via the dissimilarity matrix (P<0.001). No differences were found in the weed seed bank species composition between the G30 and G40 treatments (P>0.90) . The similarity between the weed seed bank and seedling emergence values was greater in G10 than in the G20, G30, G40 and NG treatments based on the distances of the dissimilarity matrix, where a shorter distance indicates more similarity between the emerged seedlings (i.e., vegetation) and the seed bank. Low similarity was observed for the NG treatment (Figure 2).

4. Discussion

4.1 Changes in the weed seed bank with grazing intensities in an ICLS

The soil seed bank is of particular concern to ecologists, farmers and agricultural advisors because it is the main source of seeds for future weed infestations on arable lands. Fifteen years after adopting an ICLS with different sward heights, the sizes of the weed seed banks differed from those in non-grazing areas (Figure 1). In previous research, Ikeda et al. (2007) demonstrated that using ICLS reduced the size of the weed seed bank relative to no-tillage mono-cropping systems, which is consistent with the results shown in Figure 1.

However, our results demonstrate that the process of reducing weed seed bank size when using an ICLS also depends on grazing intensity (Figure 1).

Low grazing intensities (G30 and G40) reduced the weed seed bank by 42% compared with the NG system. However, high grazing intensity (G10) increased the weed seed bank by 301% compared with the NG system. Low grazing intensity may allow taller swards to be more competitive with weeds (i.e., for light and space) than high grazing intensity, thereby reducing growth and fecundity (Baskin and Baskin, 2014) resulting as a similar ecological filter of non-grazed cover crops (McKenzie et al., 2016); some winter weeds produced seed at the end of the grazing period in the high grazing intensity (i.e., *S. brasiliensis*) that contributed to increased the soil seed banks. Conversely, N inputs from animal manure and urine may increase seed decay in the grazed treatments, reducing the longevity of the weed seeds (Davis, 2007).

4.2 Changes in seedling emergence with grazing intensities in an ICLS

Plant residues on the soil surface create a physical barrier, inhibiting seed germination and weed growth (Mohler and Teasdale, 1993), and weeds in the summer season (Table 2) represent emerged seedlings influenced by pasture residue that compete with soybean. In a related study, each centimetre of growth accounted for an additional 98 kg ha^{-1} of forage dry matter during winter, resulting in summer residues of 1.5, 3.2, 4.5 and 5.6 ton ha^{-1} in the 10-, 20-, 30- and 40-cm grazing intensity treatments, respectively (Kunrath et al., 2014). The low grazing intensities (G30 and G40) reduced the emergence of *B. plantaginea* (>93%), *R. brasiliensis* (>96%) and *S. rhombifolia* (>92%) seedlings compared to a high grazing intensity (G10) in the summer season (Table 2). It is possible that low grazing intensities resulted in a sufficient amount of pasture residue to create a physical barrier for these species; Webster et al. (2016) investigated *Amaranthus palmeri* suppression by a range of dry matter

levels of rye and found that 5370 kg ha^{-1} of rye caused a 50% reduction in the emergence of seedling for this specie. However, the surface residues can have potential allelopathic effects, and the presence of less residue under high grazing intensity may decrease the allelopathic compounds released from black oat/ryegrass (i.e., organic acids from decomposition). Additional research will be required to determine if black oat/ryegrass surface residues exert allelopathic effects on weeds.

Weeds in the winter season (Table 2) result from the emergence of seedlings that are influenced by the shade provided by the black oats/ryegrass at a given sward height, and the *C. canadensis* seedlings emerging during the winter accounted for 1.66% of the total seed bank in the G10 treatment and 0.36% of the total seed bank in the G40 treatment (Table 2). *C. canadensis* was 4.6 times more prevalent in the G10 seed bank than in the G40 seed bank, resulting in 10.7 and 0.3 emerged seedlings m^{-2}, respectively for G10 and G40 treatments (seedling emergence of *C. canadensis* in winter was 38 times more prevalent in the G10 treatment than in the G40 treatment; Table 2). Nandula et al. (2006) showed that the germination of *C. canadensis* was severely reduced in the dark (0 to 15% of germination) relative to light-exposed seeds (0 to 61% of germination) and increased the sward height increased the interception of light by forage and reduced the amount of light reaching the topsoil. In the summer season, the emergences of *C. canadensis* seedlings was 6 times more prevalent in the G10 treatment than in the G20 treatment and not found in the NG and G40 treatments, possibly due to the increased residual dry matter content present when the grazing intensity is reduced, which cover the soil and limits the amount of light that reaches the topsoil.

Tracy and Davis (2009) showed that the reduction in weed biomass in no-tillage ICLS was approximately 4.5 times higher than weed biomass in conventional systems. Our research demonstrated that areas with low grazing intensities (G30 and G40) had 2.2 times

fewer weeds m^{-2} than areas without grazing (Figure 1). De Bruijin and Bork (2006) demonstrated that managing grazing intensity provides a biological control for weeds in perennial forage via weed defoliation, which suggests that the same control can be achieved in an ICLS.

4.3 Similarity between the weed seed bank and emerged seedlings in an ICLS subjected to different grazing intensities

The similarity between the weed seed bank and seedling emergence was high in the G10 treatment and substantially lower in the G20, G30 and G40 treatments (Figure 2), potentially because the desirable forage plants maintained sufficient leaf areas in the moderate- and low-grazing intensity for the production of tillers, roots, young leaves and stems (Kunrath et al., 2014) to compete with any emerging weed seedlings. The forage in ICLS subjected to moderate- and low-grazing intensity covered the soil during the grazing period, and the resulting forage residues continued to cover the soil during the summer cropping season, which might have reduced weed recruitment from the weed seed bank (Pelissari et al., 2013).

In the weed seed banks of G10 to G40, the species richness decreased from 21.3 to 9.3 and the seedling emergence decreased from 11.3 to 2.3 and from 9.6 to 3.3 during the winter and summer, respectively (Figure 1). This observation indicates that the seed bank changed more slowly than the seedling emergence because the former was reduced by 56%, whereas the latter was reduced by 79% and 66% during the winter and summer, respectively, as the grazing intensity decreased. One explanation for this finding might be that some species with high seed dormancy and longevity characteristics remain in the seed bank for several years after a species of vegetation has disappeared (Baskin and Baskin, 2014). For example, *G. spicatum*, which dominated the weed seed bank, is a specie that is native to the

pampa rangeland (natural pastures) and covered the experimental area before the ICLS trial was implemented. As a result, the large seed bank in the soil may have resulted in part from the historical legacy effect, although we found no confirmatory reports on the longevity of *G. spicatum* in seed banks. It is also likely that incomplete weed control in the early years of the experiment (prior to glyphosate use) and the importation of *G. spicatum* seeds via wind (the seeds are dispersed as pappus-bearing achenes) contributed to the dominance of this specie in the seed bank.

Seeds of *I. purpurea* and *X. strumarium* were present in the seed banks of all treatments, but none or few emerged as seedlings (Table 2). Germination conditions for these species were not optimal until late in the season, so its late emergence (Singh et al., 2012; Northworthy 2004). In addition, when temperature conditions became optimal germination, the soybeans had established a complete canopy. The microclimate under a complete soybean canopy is obviously less favourable. However, weed emergence did not completely cease with canopy formation, and late-season weed emergence and reproduction beneath the soybean canopy may have contributed to soil seed banks. Additional study is required to determine how grazing intensities in an ICLS influence seasonal weed seedling emergence patterns and seedling recruitment at the species level.

4.4 Weed community assembly in an ICLS subjected to different grazing intensities

Weed community assembly was controlled by the grazing intensity; an ICLS with high grazing intensity had a higher Shannon's diversity index value, indicating that many rare species were present (Booth et al., 2003). The complexity of the weed community is one of the factors that cause difficulty in to planning integrated weed management and chemical weed control in an ICLS.

The species evenness was calculated using Shannon's index and varied from 0 to 1, where 0 indicates that the species community was dominated by one species (Booth et al., 2003). Our results demonstrate that grazing intensity was directly linked to the weed community structure wherein the cosmopolitan weeds (i.e., *C. canadensis*) dominated community assembly under conditions of high grazing intensity that had less evenness value (Table 1). It is Possible that high grazing intensities promote intense weed defoliation and trampling by the grazing animals, which could result in the selection of certain weeds with great plasticity and adaptability to high-intensity grazing that tolerate (i.e., through biochemical compounds that reduce tissue accessibility and palatability) or avoid (i.e., through meristem availability) the stresses imposed by animals in the winter season. In the summer season, the high grazing intensities reduce the amount of residue on the soil surface and may reduce the stress factors imposed on the establishment of cosmopolitan weeds (i.e., reduced the potential allelopathic effects and the physical barrier of surface residues).

The grazing intensities did not affect the weed seed bank or seedling emergence of either *H. reniformis* or *P. tenellus* (Table 2). Some weed species in the seed bank and aboveground vegetation disappeared as the grazing intensity decreased (Table 2; i.g., *C. esculentus* was found only in G10 treatment). We did not measure annual weed population dynamics (seed rain inputs) directly; therefore, were unable to determine the rate of disappearance of some species or species invasion, and the present data can support only a speculative discussion. Additional research is necessary to determine why some weed species disappeared.

4.5 Implications of different grazing intensities for weed management in an ICLS

Our study considers an area of great interest for world food security, and the results have several potential implications for integrated weed management beyond the possible use

of grazing intensities as a tool for biological weed control. For example, minimizing grazing intensities reduced seedlings emergence of *C. canadensis* and *S. brasiliensis* in winter and *B. plantaginea*, *R. brasiliensis* and *S. rhombifolia* in summer (Table 2).

We analysed the costs of field desiccation before planting soybeans and found that different weed species compositions occurred among the treatments (Table 2), which would also require different control methods (following Rodrigues and Ameida, 2011). Treatment G10 contained *R. brasiliensis* and *P. tomentosa*, which require approximately 2,400 g a.i. ha^{-1} of glyphosate for control, whereas treatment G20 contained *S. americanum*, which requires approximately 960 g a.i. ha^{-1} of glyphosate for control. Treatments G30, G40 and NG require approximately 480 g a.i. ha^{-1} of glyphosate for weed control. The cost of 480 g a.i. of glyphosate is approximately 4.15 US$.

Furthermore, treatments G10, G20, and G30 contained *C. canadensis* populations above the economic threshold during the winter (assuming threshold similar to that of *C. bonariensis* for soybeans as defined by Trezzi et al., 2015). *C. canadensis* has developed glyphosate-resistance and can severely reduce soybean production (Beckie, 2011). The cost of controlling glyphosate-resistant *C. canadensis* can reach approximately 45 US$ ha^{-1} (Trezzi et al., 2015). In the G10 treatment, *C. canadensis* required control beyond winter desiccation because the density of the emerged *C. canadensis* seedlings in the summer crop was high (Table 2) and requires multiple herbicide applications for control (Trezzi et al., 2015). When considering only weed-control costs, the most economical treatments were the G40 and NG treatments, with herbicide costs of approximately 4.15 US$ ha^{-1}, whereas the G20 and G30 treatments had a herbicide costs of 53.3 US$ ha^{-1}, and the G10 treatment had a herbicide cost of approximately 110.75 US$ ha^{-1} (Trezzi et al., 2015). When conducting an economic analysis of this protocol, Oliveira et al. (2013) found overall gross margins of 890, 861, 712, 643 and 431 US$ (corrected for the current price of 1 US$ = 4 Brazilian reals) for

G10, G20, G30, G40 and NG, respectively. In this case, however, the authors assumed that the costs for weed control were equal among the treatments. Based on our research results and after adjusting the gross margin as a function of the reduced cost of weed control, we found gross margins of 890, 918, 769, 819 and 538 US$ for the G10, G20, G30, G40 and NG treatments, respectively. This relatively simple economic analysis of the ICLS suggests that the optimal grazing intensity treatment for attaining economic goals was the G20 treatment, despite the lower weed control cost of the G40 treatment.

Considering current discussions of the sustainability of glyphosate and the increased resistance of weeds to glyphosate (Busi et al., 2013), these results demonstrate that low grazing intensities (G40) require lower glyphosate application rates than higher grazing intensities (G10) because of the weed species that are present. Beckie (2011) showed that increasing glyphosate application rates accelerates the process by which weeds become glyphosate resistant. Thus, using low grazing intensities in ICLS could improve the temporal sustainability of glyphosate. Furthermore, Davis et al. (2006) indicate that improving our ability to reduce the weed seed bank is critical for integrated weed management. This research suggests that using low grazing intensities in ICLS is a form of biological control that can be used to reduce the weed seed bank in the cropping areas of the ICLS (Figure 1); more specifically, minimizing grazing intensities reduces seeds in the soil seed banks of *B. plantaginea*, *C. canadensis*, *G. spicatum*, *P. tomentosa*, *R. brasiliensis*, *S. brasiliensis* and *S. rhombifolia* (Table 2).

5. Conclusion

In an ICLS, the grazing intensity significantly affects the emergence of weed seedlings and the size of the weed seed bank. Specifically, decreasing the grazing intensity to enable greater sward height in winter-grazed cover crops reduces the number of weed

species, the density of emerged weed seedlings, and the weed seed bank size. Integrated weed management strategies should consider grazing intensities in ICLS.

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7. References

Baskin, C.C., Baskin, J.M., 2014. Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination, Seconded. Academic Press, San Diego, CA.

Beckie, H.J., 2011. Herbicide-resistant weed management: focus on glyphosate. Pest Manag. Sci. 67, 1037–1048. doi:http://dx.doi.org/10.1002/ps.2195.

Bolliger, A., Magid, J., Amado, T.J.C., Neto, F.S., Ribeiro, M.F.S., Calegari, A., Ralsch, R., Neergaard, A. 2006. Taking stock of the Brazilian "zero-till revolution": a review of landmark research and farmers' practice. Adv. Agron., 91, 47-64. doi:10.1016/S0065-2113(06)91002-5

Booth, B.D., Murphy, S.D., Swanton, C.J., 2003. Weed Ecology in Natural and Agricultural Systems. CABI Publication, Wallingford, Oxon, UK.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol. Monogr. 27, 235-349. doi:http://dx.doi.org/10.2307/1942268.

Busi, R., Vila-Aiub, M.M., Beckie, H.J., Gaines, T.A., Goggin, D.E., Kaundun, S.S., Lacoste, M., Neve, P., Nissen, S.J., Norsworthy, J.K., Renton, M., Shaner, D.L., Tranel, P.J, Wright, T., Yu, Q., Powles, S.B. 2013. Herbicide-resistant weeds: from research and

knowledge to future needs. Evol. Appl. 8, 1218-1221. doi:http://dx.doi.org/10.1111/eva.12098.

Cardina, J., Herms, C.P., Doohan, D.J., 2002. Crop rotation and tillage system effects on weed seedbanks. Weed Sci. 50, 448-460. doi:http://dx.doi.org/10.1614/0043-1745(2002)050[0448:CRATSE]2.0.CO;2.

Carvalho, P.C.F., Franzlubbers, A.J., Lemaire, G., Sulc, M.R., Dedieu, B., 2014. Towards agricultural sustainability through integrated crop-livestock systems. II. Production responses. Renewable Agric. Food Syst. 29, 192-194. doi:http://dx.doi.org/10.1016/j.agee.2014.04.028.

Davis, A.S., 2007. Nitrogen fertilizer and crop residue effects on seed mortality and germination of eight annual weed species. Weed Sci. 55, 123-128. doi:http://dx.doi.org/10.1614/WS-06-133.1.

Davis, A.S., Anderson, K.I., Hallett, S.G., Renner, K.A., 2006. Weed seed mortality in soils with contrasting agricultural management histories. Weed Science 54, 291-297. doi:http://dx.doi.org/10.1043/0043-1745(2006)54[291:WSMISW]2.0.CO;2.

De Bruijin, S.L., Bork, E.W., 2006. Biological control of Canada thistle in temperate pastures using high density rotational cattle grazing. Biol. Contr. 36, 305–315. doi:http://dx.doi.org/10.1016/j.biocontrol.2005.10.007.

De Cauwer, B., Van den Berge, K., Cougnon, M., Bulcke, R., Reheul, D., 2010. Weed seedbank responses to 12 years of applications of composts, animal urines or mineral fertilizers. Weed Res. 50, 425–435. doi:http://dx.doi.org/10.1111/j.1365-3180.2010.00796.x.

Díaz, S., Lavorel, S., McIntyre, S., Falczuk, V., Casanoves, F., Milchunas, D.G., Skarpe, C., Rusch, G., Sternberg, M., Noy-Meir, I., Landsberg, J., Zhang, W., Clark, H.,

Campbell, B.D., 2007.Plant trait responses to grazing - a global synthesis. Glob. Change Biol. 13, 313-341. doi:http://dx.doi.org/10.1111/j.1365-2486.2006.01288.x.

Franzluebbers, A.J., Lemaire, G., de Faccio Carvalho, P.C., Sulc, R.M., Dedieu, B., 2014. Toward agricultural sustainability through integrated crop–livestock systems. III. Social aspects. Renew. Agric. Food Syst. 29, 192-194. doi:http://dx.doi.org/10.1017/S174217051400012X.

Haynes, R.J., Willianms, P.H., 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Adv. Agron. 49, 119-199.

Ikeda, F.S., Mitja, D., Vilela, L., Carmona, R., 2007. Banco de sementes no solo em sistemas de cultivo lavoura-pastagem. Pesq. Agropec. Bras. 42, 1545–1551. doi:http://dx.doi.org/10.1590/S0100-204X2007001100005.

Kent, M., Coker, P., 1992. Vegetation Description and Analysis: a Practical Approach. Belhaven Press, London.

Keulen, H., Schiere, H., 2004. Crop-Livestock system: old wine in new bottles? In: Fisher, T. et al.(Eds.), New Directions for a Diverse Planet: Proceedings of the IV International Crop Science Congress. Australia.

Kissmann, K.G., Groth, D., 1997, Plantasinfestantes e nocivas.BASF, São Paulo. Obra em 3 volumes.

Kunrath, T.R., Cadenazzi, M., Brambilla, D.M., Anghinoni, I., Moraes, A., Barro, R.S., Carvalho, P.C.F., 2014. Management targets for continuously stocked mixed oat X annual ryegrass pasture in a no-till integrated crop-livestock system. Eur. J. Agron. 57, 71-76. doi:http://dx.doi.org/10.1016/j.eja.2013.09.013.

Lemaire, G., Franzluebbers, A., Carvalho, P.C.F., Dedieu, B., 2013. Integrated crop–livestock systems: strategies to achieve synergy between agricultural production and

environmental quality. Agr. Ecosyst. Environ. 190, 4-8. doi:http://dx.doi.org/10.1016/j.agee.2013.08.009.

Lorenzi, H.M., de Identificação e Controle de Plantas daninhas, sixth ed. Nova and Publishing House, Odessa, São Paulo: Instituto Plantarum, 2006.

Ma, M., Zhou, X., Ma, Z., Du, G., 2012. Composition of the soil seed bank and vegetation changes after wetland drying and soil salinization on the Tibetan Plateau. Ecol. Eng. 44, 18-24. doi: http://dx.doi.org/10.1016/j.ecoleng.2012.03.017.

Ma, Z., Ma, M., Baskin, J.M., Baskin, C.C., Li, J., Du, G. 2014. Responses of alpine meadow seed bank and vegetation to nine consecutive years of soil fertilization. Ecol. Eng. 70, 92-101. doi: http://dx.doi.org/10.1016/j.ecoleng.2014.04.009.

McKenzie, S.V., Goosey, H.B., O'Neill, K.M., Menalled, F.D. 2016. Impact of integrated sheep grazing for cover crop termination on weed and ground beetle (Coleoptera: Carabidae) communities. Agric. Ecosyst. Environ. 218, 141-149. doi: http://dx.doi.org/10.1016/j.agee.2015.11.018.

Mohler, C.L., Asdale, J.R., 1993. Response of weed emergence to rate of *Vicia villosa* Roth and *Secale cereale* L. Residue. Weed Res. 33, 487-499. doi:http://dx.doi.org/10.1111/j.1365-3180.1993.tb01965.x.

Moraes, A.d, Carvalho, P.C.dF., Lustosa, S.B.C., Lang, C.R., Deiss, L., 2014. Research on integrated crop-livestock systems in Brazil. Rev. Ciênc. Agron. 45, 1024–1031. doi:http://dx.doi.org/10.1590/S1806-66902014000500018.

Neto, A.B., Savian, J.V., Schons, R.M.T., Bonnet, O.J.F., Canto, M.W., Moraes, A., Lemaire, G., Carvalho, P.C.F., 2014. Italian ryegrass establishment by self-seeding in integrated crop-livestock systems: Effects of grazing management and crop rotation strategies. Eur. J. Agron. 57, 77-83. doi:http://dx.doi.org/10.1016/j.eja.2014.04.005.

Norsworthy, J.K., 2004. Soybean canopy formation effects on pitted morningglory (*Ipomoea lacunosa*), common cocklebur (*Xanthium strumarium*), and sicklepod (*Senna obtusifolia*) emergence. Weed Science. 52, 954-960. doi:http://dx.doi.org/10.1614/WS-04-043R.

Oliveira, C.A.O., Bremm, C., Anghinoni, I., Moraes, A., Kunrath, T.R., de Faccio Carvalho, P.C., 2013. Comparison of an integrated crop-livestock system with soybean only: economic and production responses in southern Brazil. Renew. Agric. Food Syst. 29, 230-238. doi:http://dx.doi.org/10.1017/S1742170513000410.

Pelissari, A., Victoria, R., Mendonça, C.G., Lustosa, S.B.C., Marques, P.F., 2013. Fundamentação teórica para o controle de plantas daninhas em integração lavoura-pecuária. Silva, J. F. & Martins, D. Manual de aulas práticas de plantas daninhas. Funep, Jaboticabal, pp. 31-44.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simonds, C., O`Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T., Tsomondo, T. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. Agric. Ecosyst. Environ. 84, 1-20. doi:http://dx.doi.org/10.1016/S0167-8809(00)00178-X.

Robertson, G.P., Swinton, S.M., 2005. Reconciling agricultural productivity and environmental integrity: a grand challenge for agriculture. Front. Ecol. Environ 3, 38-46. doi:http://dx.doi.org/10.1890/1540-9295(2005)003[0038:RAPAEI]2.0.CO;2.

Rodrigues, B.N., Almeida, F.S., 2011. Guia de herbicidas. 6. Grafmarke, Londrina.

Singh, M., Ramirez, A.H.M., Sharma, S.D., Jhala, A.J. 2012. Factors affecting the germination of tall morningglory (*Ipomoea purpurea*). Weed Sci., 60, 64-68. doi: http://www.bioone.org/doi/full/10.1614/WS-D-11-00106.1

Soil Survey Staff, 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys, in: USDA Natural Resource Conservation Service Agriculture Handbook No. 436. U.S. Government Printing Office, Washington, DC.

Thompson, K., Bakker, J.P., Bekker, R.M., 1997, The Soil Seed Banks of North West Europe: Methodology, Density and Longevity. Cambridge University Press, Cambridge, UK.

Tracy, B.F., Davis, A.S., 2009. Weed biomass and species composition as affected by an integrated crop–livestock system. Crop Sci. 49, 1523-1530. doi:http://dx.doi.org/10.2135/cropsci2008.08.0488.

Trezzi, M.M., Vidal, R.A., Patel, F., Miotto, E., Debastiani, F., Balbinot Jr., A.A., Mosquen, R., 2015. Impact of *Conyza bonariensis* density and establishment period on soybean grain yield, yield components and economic threshold. Weed Res. 55, 34–41. doi:http://dx.doi.org/10.1111/wre.12125.

Webster, T.M., Simmons, D.B., Culpepper, A.S., Grey, T.L., Bridges, D.C., Scully, B.T. 2016. Factors affecting potential for Palmer amaranth (*Amaranthus palmeri*) suppression by winter rye in Georgia, USA. Field Crops Res. 192, 103-109. doi: http://dx.doi.org/10.1016/j.fcr.2016.04.020.

Wiles, L., Schweizer, E., 2002. Spatial dependence of weed seed banks and strategies for sampling. Weed Sci. 50, 595-606. doi:http://dx.doi.org/10.1614/0043-1745(2002)050[0595:SDOWSB]2.0.CO;2.

Tables

Table 1
Analysis of Shannon's diversity index and species evenness at different grazing intensities

Grazing intensities	Shannon index			Species evenness		
	Vegetation		Seed bank	Vegetation		Seed bank
	Winter	Summer		Winter	Summer	
10*	1.70 b	1.84 c	2.44 c	0.46 a	0.57 a	0.54 a
20	0.97 ab	1.65 bc	2.03 a	0.65 ab	0.75 b	0.49 a
30	0.43 a	1.46 b	1.87 a	0.85 bc	0.76 bc	0.75 c
40	0.69 a	1.16 a	1.96 a	0.96 c	0.88 c	0.77 c
NG	-	1.26 a	2.25 b	-	0.71 b	0.61 b

In each column, means with same letter do not differ at the 0.05 probability level according to LSD testing.
* Sward height (cm); NG = non-grazed

Table 2
Species population density changes in seedling emergence (seedlings m^{-2}) and seed banks (seeds m^{-2} at 0-5cm soil depth) as affected by grazing intensity and in non-grazed areas

Species		Seedling emergence										Seed bank				
		Winter					Summer									
	LF*	NG**	10***	20	30	40	NG	10	20	30	40	NG	10	20	30	40
Brachiaria plantaginea (Link) Hitchc.	A		****0.19 a			0.09 a	1.65 a	2.71 a	1.48 a	0.19 bc	0.14 c	96.4 b	155.3 a	92.4 b	31.4 c	48.0 c
Conyza bonariensis (L.) Cronquist	A		0.05										38.4 a	19.2 a		
Conyza canadensis (L.) Cronquist	A		10.7 a	2.5 b	0.83 c	0.28 d		1.90 a	0.30 b	0.14 b		159 b	643.2 a	336.0 b	96.0 c	76.8 c
Cyperus esculentus L.	P							0.05 a			0.05 a		28.8			
Digitaria horizontalis Willd.	A		0.09				0.63 a	0.81 a	0.43 a	0.05 b		49.4 a	38.8 a			
Echium plantagineum L.	A		0.28 a	0.05 a									35.8 a	28.8 a		
Eleusine indica L. Gaertn	P		0.05				0.18 a	0.57 a			0.05 b	63.2 a	27.0 a	38.3 a	28.3 a	38.4 a
Facelis retusa (Lam.) Schultz-Bip.	A		0.71										67.2			
Gnaphalium spicatum Lam.	A		0.23 a		0.05 a							433.7 b	729.6 a	518.4 ab	163.2 c	150.5 c
Heteranthera reniformis Ruiz &Pav.	P		0.62 a	0.19 a	0.17 a			0.62 a	0.05 a	0.38 a	0.52 a	14.4 a	9.6 a	9.6 a		
Ipomoea purpurea (L.) Roth	A										0.05	57.6 b		96.0 a	57.6 a	48.0 a
Nothoscordum gracile (Aiton.) Steam.	P		0.14 a			0.09 a							97.6 a	11.2 b		
Phyllanthus tenellus Roxb.	A		0.28 a	0.33 a				0.04 a	0.28 a	0.09 a		27.3 a	32.1 a	28.8 a		
Plantago tomentosa Lam.	A		2.38					0.24				35.7 b	124.8 a	27.2 b	29.7 b	38.4 b
Richardia brasiliensis Gomes	A		0.86				0.39 a	4.81 a	0.28 ab	0.14 b		40.0 b	604.8 a	28.8 b	27.1 b	38.4 b
Rumex obtusifolius L.	P															9.6
Senecio brasiliensis Less.	P		0.76 a	0.57 a	0.05 b			0.23 a	0.10 a	0.04 a		60.5 b	124.8 a	48.0 b	9.6 b	19.2 b
Sida rhombifolia L.	P							0.67 a	0.29 ab	0.05 b	0.05 b	78.8 a	86.4 a	28.8 b	29.4 b	25.5 b
Solanum americanum Mill.	A		0.05 a	0.05 a				0.05 a	0.19 a			29.4 a	27.0 a	25.8 a		
Soliva pterosperma (Juss.) Less.	A		3.57									33.2 b	144.0 a	48.0 b		
Stachys arvensis L.	A		0.33 a	0.05 a								16.1 b	211.2 a	19.2 b		
Stellaria media (L.) Vill.	A												403.2 a			9.6 b
Taraxacum officinale F.H. Wigg.	P		0.67										42.5			
Xanthium strumarium L.	A						0.21 a	0.05 a		0.05 a		32.5 a	28.0 a	29.2 a	9.6 a	

*LF = Life cycle (A = annual; P = perennial); **NG = non-grazed; *** Sward height (cm) during the pasture phase; ****For each variable, means within a time period (winter or summer) of the seed bank data followed by the same letters and the same lowercase letters in a column do not differ according to LSD testing ($p<0.05$) for homogeneous data and according to Mann-Whitney U-testing ($p<0.05$) for non-homogeneous data. Blank fields indicate that the species was not found.

Figure captions

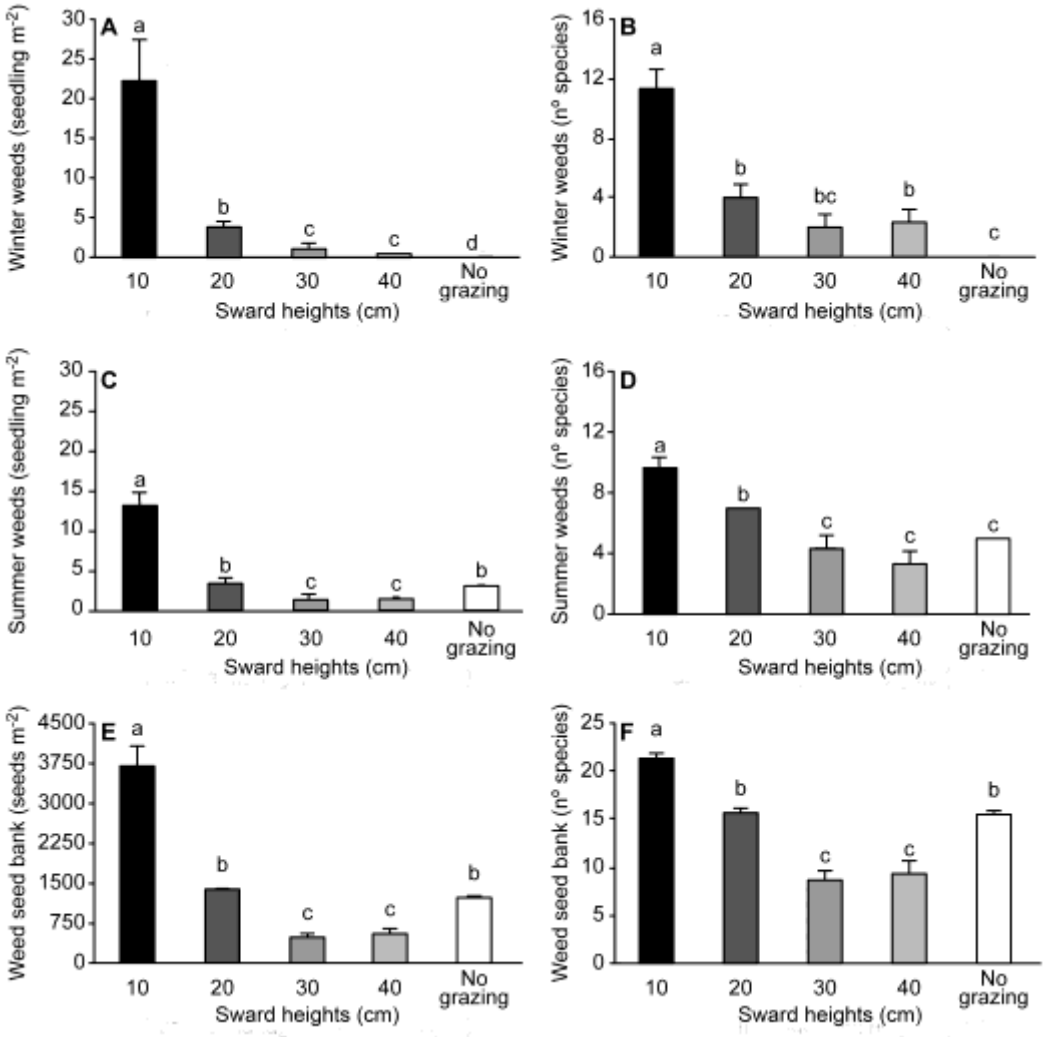

Figure 1. Changes in winter (a,b) and summer (c,d) weed seedling emergence density (seedlings m^{-2}; a,c), species richness (n^o species; b,d), weed seed bank (e,f) density (seeds m^{-2} at 0-5 cm soil profile; e), and richness (n^o species; f) after 15 years under different grazing intensities in an ICLS in Southern Brazil. Data points with different lowercase letters indicate significant differences among the treatments according to the LSD test ($P<0.05$) for species richness and the Mann-Whitney U test ($p<0.05$) for density (m^{-2}). Columns represent means, and error bars represent standard deviations.

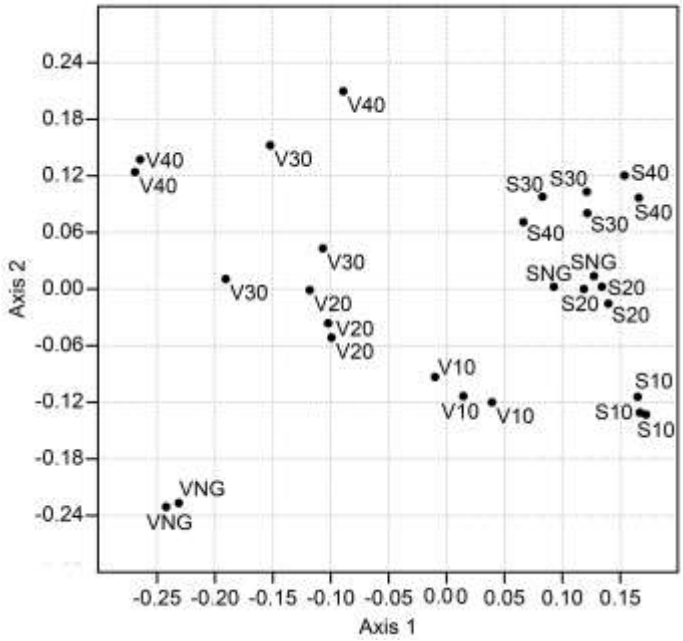

Figure 2. Non-metric multidimensional scaling (NMDS) ordination plots based on the species compositions of seedling emergence (winter+summer) and the seed bank in an ICLS. Stress value=0.1566. Ordinations based on the Bray-Curtis dissimilarity matrix for relative abundance. V: seedling emergence (vegetation), S: seed bank, and NG: non-grazing. Numbers indicate the sward heights (cm).

CHAPTER 2

Effects of crop rotation and sheep grazing management on the seed bank and emerged weed flora under a no-tillage integrated crop-livestock system[1]

[1] Prepared in accordance with the standards of the *The Journal of Agricultural Science*.

Effects of crop rotation and sheep grazing management on the seed bank and emerged weed flora under a no-tillage integrated crop-livestock system

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Effects of crop rotation and sheep grazing management on the seed bank and emerged weed flora under a no-tillage integrated crop-livestock system

Abstract

Effective integrated weed management in agricultural landscapes depends on ability to identify and manage processes that drive weed dynamics. This study reports the effects of grazing management and crop rotation strategies on the seed bank and emerged weed flora in an integrated crop-livestock system (ICLS) experiment during a 12-year period under no tillage in subtropical southern Brazil. During winter, Italian ryegrass cover crops were grazed by sheep, and the grazing management treatments included two stocking methods (continuous and rotational) and two forage allowances (10 and 20 kg of herbage dry matter available 100 kg/ha per animal live weight). During summer, the crop rotation treatments involved either soybean-maize or soybean-soybean in succession with winter-grazed cover crops. The treatments were part of a factorial randomized complete block design. We evaluated treatment effects on the weed seed bank and emerged weed flora populations during winter-grazed cover crop and summer crop growth as well as during the harvest phase. The current results demonstrate that crop rotation and grazing management exhibited interactive effects on the determination of weed outcomes in an ICLS. However, overall, compared with moderate forage allowance, high forage allowance during the winter-grazed cover crop caused lower emerged weed flora in subsequent crops (i.e., a 20% reduction during crop growth and a 90% reduction at crop harvest) and a 48% reduction in seed bank size. High forage allowance promoted more residue of winter-grazed cover crop biomass that remained during the summer crop phases and likely resulted in a physical barrier to weed emergence.

Introduction

Agricultural production on existing land must feed the world population in a sustainable manner (Robertson & Swinton 2005; Fedoroff *et al.* 2010). Ongoing research on integrated crop-livestock systems (ICLSs) throughout the world has shown promise for providing synergy between agricultural production practices and environmental quality (Herrero *et al.* 2010; Lemaire *et al.* 2014).

Weeds remain a major constraint to productivity, and the increasing prevalence of herbicide-resistant weeds worldwide is alarming in terms of global food security, as the majority of agricultural systems rely heavily on herbicides for weed control (Busi *et al.* 2013). Research is being conducted on a variety of cropping systems to develop more effective integrated weed management methods that are ultimately sustainable and reduce reliance on herbicides (Lechenet *et al.* 2017). Investigations into the effects of including livestock grazing in crop-based systems have demonstrated that, compared with conventional cropping systems, ICLSs can result in lower amounts of weed infestation as well as lower costs and risks of herbicides (Tracy & Davis 2009; Miller *et al.* 2015; Lehnhoff *et al.* 2017). However, these investigations revealed that, in some years, livestock led to an increase in weed infestation in arable lands (Miller *et al.* 2015).

The effectiveness of grazing for weed control depends on preferences, the type of pasture, the duration of grazing, and the number of grazing animals (Lacey & Sheley 1996; De Bruijn & Bork 2006; Renne & Tracy 2013; Lustosa *et al.* 2016). Additionally, many studies have demonstrated the benefits of diversified crop rotation for reducing weed infestation in arable crops (e.g., Cardina *et al.* 2002; Bellinder *et al.* 2004; Hosseini *et al.* 2014). In ICLSs, these factors (i.e., grazing management and crop rotation) can occur over the same area at different spatial-temporal scales within a year. For example, the practice of using a field for grazing a grass cover crop in the winter and producing row crops the following summer is widespread in subtropical and temperate regions of the USA (Sulc &

Franzluebbers 2014), Brazil (De Moraes *et al*. 2014) and Australia (Nie *et al.* 2016); however, information is lacking on how grazing management and crop rotation collectively impact weed population dynamics and affect weed outcomes in ICLSs.

The current study objectives were to assess the effects of crop rotation, forage allowance, and animal stocking method on the seed bank and emerged weed flora in an ICLS. In addition, it was analyzed how these management practices differ in their effects on weed community composition.

Materials and methods

Site and treatment descriptions

This study was conducted in a long-term ICLS experiment under no-tillage management and was located at the research farm of the Federal University of Rio Grande do Sul in Rio Grande do Sul State, Brazil (30°05' S; 51°39' W). The experimental site covers a total area of 4.8 ha, and the summers are warm and humid (Cfa according to the Köppen classification system). The study area is characterized by a marked seasonality of temperature and a fairly homogeneous distribution of precipitation throughout the year (Neto *et al.* 2014). The soil is classified as a Typic Paleudult (United States Department of Agriculture [USDA] 1999); the soil within a depth of 0-20 cm contained 15% clay and 2.0% organic matter, and the pH was 4.87.

Before 2003, native pasture (pampa rangeland) covered the experimental area. Beginning in 2003, the area was converted into no-tillage cropland, and an ICLS experimental protocol was established that consisted of two growing seasons per year: (1) a winter season with cover crop pasture grazed by sheep from May until November and (2) a summer season with row crop production from November until April.

In March of 2003 and 2004, glyphosate was applied at 2400 g ae/ha to eliminate all vegetation prior to seeding Italian ryegrass (*Lolium multiflorum* Lam. cv. "Common" at 45 kg seed/ha) for the winter grazing season. Since 2005, naturally reseeded Italian ryegrass has formed the winter pasture, and the pasture establishment phase has occurred from late May until late June, which is the common practice for ICLSs in the region (Neto *et al.* 2014). In early July, the stocking phase was initiated with the introduction of sheep for the winter grazing period. The ryegrass becomes reproductive and sheds seed at the end of each winter grazing period from late October through November. Treatment factors included stocking method (continuous or rotational), forage allowance (moderate or high), and summer cropping (continuous soybean or soybean-maize rotation). Treatments consisted of a 2 by 2 by 2 factorial in a randomized complete block design; four replicates were included, totaling 32 experimental units. The plot size was 1400/m^2 (20 by 70 m). The forage allowance treatments were defined as 2.5 times (moderate forage allowance) and 5 times (high forage allowance) the potential daily dry matter intake of lambs in accordance with the National Research Council [NRC] (1985), resulting in 10 kg and 20 kg of forage dry matter 100 kg/ha per animal live weight for the moderate and high forage allowance treatments, respectively. For the continuous stocking treatment, the entire plot received three tester animals (animals that remained permanent throughout the grazing period) plus a variable number of animals periodically adjusted with put-and-take animals that were added or removed from the plot as required to maintain the desired forage allowance. For rotational stocking, it was divided the plots into successive grazing tracts with an electric fence adjusted to maintain a minimum of three animals in each plot for the desired forage allowance. The length of the grazing cycle in each grazing tract was previously defined as a function of Italian ryegrass leaf lifespan (500 and 410 growing degree days [GDDs] in August and September-October, respectively (Pontes *et al.* 2003)) to ensure that leaf senescence was minimized in the grazing tract before the

animals returned.

At the end of the winter season in late November, the pasture vegetation was treated with glyphosate (2400 g ae/ha), and the residual biomass was approximately 3.2 and 1.8 ton/ha for the high and moderate forage allowance, respectively. The row crop treatments were sown at an inter-row spacing of 0.4 m in December of each year. In the first two years of the experiment, conventional non-transgenic soybeans were sown, and a transgenic glyphosate-resistant cultivar was sown in subsequent years. A conventional non-transgenic maize hybrid was sown in the first ten years of the experiment, and a transgenic glyphosate-resistant hybrid was sown in subsequent years. In the first two years, the conventional soybeans received a postemergence application of imazethapyr (150 g ai/ha) and tepraloxydim (100 g ai/ha) in mid-January, and the conventional maize received a postemergence application of tembotrione (100 g ai/ha) or nicosulfuron (60 g ai/ha) during the first ten years. As a postemergence herbicide, glyphosate at 2400 g ae/ha was applied to the transgenic glyphosate-resistant soybean and maize in mid-January. The application volume was 200±50 l/ha, and adjuvants were not used. Insecticides and fungicides were applied as needed in accordance with agronomic recommendations. Row crops were harvested at the end of May each year.

Seed bank sampling

Soil seed banks were sampled before summer crop sowing in November 2014. A total of 28 soil cores were taken within the central 4 by 52-m area of each plot. The soil samples were collected manually at the intersections of a 4 by 4-m grid using a steel 4.2-cm-diameter probe inserted to a depth of 10 cm. Each core was divided into 0-5-cm- and 5-10-cm-depth segments.

The 28 soil cores for each sampling depth within each plot were bulked and mixed. The

bulked samples for each plot (minus large stones and root fragments) were spread out in 48-cm by 38-cm plastic trays to record seedling emergence. The plastic trays were kept for 12 months in a greenhouse, and the seedling emergence method (Thompson *et al.* 1997) was used to quantify readily germinable seeds from the seed banks. The measured active seed bank closely reflected the total viable seed bank, and this phenomenon was affirmed by squeezing the non-germinated seeds recovered from two randomly chosen test trays; only 2% of the larger seeds remained firm when squeezed with forceps. Optimum soil moisture conditions in the trays were maintained by regular sub-irrigation (i.e., three times per week). In the winter, the lowest night-time temperature in the greenhouse was 10 °C, and the maximum daytime temperature was 30 °C. Emerged seedlings were identified, counted, and removed from the plastic trays weekly. A two-week drought period was imposed in March 2015 to break seed dormancy (Cauwer *et al.* 2010). At the end of the drought period, the contents of the trays were stirred and sub-irrigation was reactivated. After seed emergence ceased, the samples were stirred and placed in a 4 °C cold room for 3 weeks followed by 1 week of alternating temperatures (15 and 4 °C) before being returned to the greenhouse (Cardina *et al.* 2002). This process was repeated until no additional seedlings emerged.

Sampling of weed flora

In each field plot, emerged weed flora was determined at three times during each year: at the end of the grazing season (November 2014 and 2015), before herbicide applications for soybean or maize cultivation (mid-January 2015 and 2016), and after crop harvest (May 2015 and 2016). Emerged weed flora was determined just before the point when herbicides are typically applied or when other interventions for weed control occur in this type of ICLS (in this protocol, only herbicides were used to control weeds). At the end of the winter grazing season, the area was treated with glyphosate to introduce grain crops. During the summer crop

growing season, herbicides were used to control weeds to avoid weed interference, and after harvest, herbicides were applied to ensure pasture establishment. In the central area (4 by 52 m) of each plot, the emerged weed flora within 50-cm by 50-cm quadrats located at the intersections of a 4-m by 10-m grid were counted and identified, resulting in 10 subsamples per plot. The weeds were identified in accordance with the methods of Kissmann & Groth (1997) and Lorenzi (2006). The means of the 10 subsamples were used for statistical analysis in accordance with the suggestion of Onofri *et al.* (2010), and the data were reported as the population density (weeds/m^2) for each species.

Data analyses

Weed density was evaluated first using a global analysis of variance (ANOVA) with a generalized linear model (GLM); year, block, treatment (stocking method, forage allowance and crop rotation), sampling time (grazing, crop, and harvest) and previous crop (maize or soybean) were designated as main effects. All interactions between the previous crop, year and sampling time with treatments were also tested. In all cases, no significant interactions among treatment and year or the previous crop were detected (P>0.05), and a stepwise backward elimination of terms was used based on the Akaike Information Criterion (AIC) to confirm that the year and previous crop did not influence the weed density analysis. Sampling time exhibited significant interactive effects with treatment, and the data were analyzed separately for each time. Thus, for weed density at each time (i.e., grazing, crop, and harvest) and for the weed seed bank size at each depth, the ANOVA were conducted using linear mixed-effects models that outperformed other models; blocks were designated as random effects, whereas crop rotation, stocking method, and forage allowance were designated as fixed effects. The model was selected based on the AIC. Differences among treatments were tested using the Tukey HSD test (P<0.05). Treatment effects at the species level were

detected using multivariate analysis of variance (MANOVA); the models included terms for year and the previous crop as well as their interactions with treatments. No significant interactions among treatments or between treatments and year or the previous crop were detected (P>0.05). Thus, multiple ANOVAs with a single treatment factor were performed for each species. The total weed density and species density data were log transformed to normalize the variances, and the normality and homogeneity were tested by using the Shapiro-Wilk test (P>0.05) and the Bartlett test (P>0.05), respectively. Due to the large number of species (Table 1), the results presented at the species level in the tables are focused on the most prevalent species (relative density>0.5 plants/m^2 for the emerged weed flora and >100 seeds/m^2 for the seed bank) for which significant treatment effects were observed.

The weed community composition and structure were calculated by considering all emerged weed flora (summing all sampling times of emerged weed flora, i.e., winter-grazed cover crop+summer crop and harvest phase) in order to represent the total weed community of the systems (rather than for each season). To test the effects of grazing management (forage allowance and stocking) and crop rotation on weed community composition, we used non-metric multidimensional scaling (NMDS) ordination for the visual representation of community differences. Bray-Curtis dissimilarity indices (Bray & Curtis 1957) were calculated based on the following equation: $BC_{jk}=\sum^{s}_{i=1}(2a_{ij}-a_{ik})/\sum^{s}_{i=1}2a_{ij}+\sum^{s}_{i=1}2a_{ik}$, where BC_{jk} is the dissimilarity between sites j and k; a_{ij} and a_{ik} are the relative species densities of species i at sites j and k, respectively; and S is the combined total density of the species in both communities. The data were log-transformed to de-emphasize the effect of dominant species using the following equation: $T_{ij}=\log_2(M_{ij}+1)$, where T_{ij} is the log-transformed density of species i in community j and M_{ij} is the raw density of species i in community j (McKenzie *et al.* 2016). Bray-Curtis dissimilarities were subjected to permutation-based multivariate analysis of variance (PerMANOVA), which included terms for blocks to test for grazing

management (allowance and stocking) and crop rotation effects on the weed seed bank and emerged weed flora community composition (McCune *et al.* 2002). The Shannon diversity index (H) of the weed seed banks and emerged weed flora for each treatment were estimated using the following equation: $H=\sum^{s}_{i=1}(ni/N)(\log_2 ni/N)$, where *N* is the total number of individuals per plot, *ni* refers to the number of individuals per species per plot and *S* describes the total number of species. The evenness (J) of the species in each treatment was also calculated using the Shannon diversity index, where $J=H/\log_2(s)$, as described by Hosseini *et al.* (2014). The species richness, Shannon diversity, and evenness data were subjected to ANOVA using linear mixed-effects models; blocks were designated as random effects, whereas crop rotation, stocking method, forage allowance and years were designated as fixed effects. Model selection was based on the AIC. The normality and homogeneity were tested by using the Shapiro-Wilk test ($P>0.05$) and the Bartlett test ($P>0.05$), respectively. All analyses were conducted using R Version 3.1.0 (© 2014 The R Foundation for Statistical Computing).

Results

Weed seed bank size and vertical distribution

The weed seed banks near the soil surface were affected by forage allowance but were similar among crop rotation and stocking treatments. Forage allowance significantly affected the overall weed seed bank density at the 0-5-cm and 0-10-cm soil depths ($P<0.05$). However, no significant treatment differences were observed in the seed bank at the 5-10-cm soil depth ($P=0.33$). Compared with the moderate forage allowance (high stocking rates), high forage allowance (lower stocking rates) reduced the weed seed density. Furthermore, compared with moderate forage allowance, high forage allowance led to a 48% lower overall seed bank

density in the top 5 cm of the soil (Fig. 1). No significant differences in the overall weed seed bank size were found for crop rotation or stocking treatments (P=0.27 and P=0.58, respectively). At the species level, the total amount of seeds of some species was affected at the 0-5-cm soil depth (P<0.05; Table 2) but not at the 5-10-cm soil depth (P>0.05). The effects of forage allowance on the total amount of weed seeds appear to be driven by species-specific responses. An approximately 10-fold greater seed density for both *C. bonariensis* and *S. media* was observed under moderate forage allowance than under high forage allowance. Other seed bank species in Table 1 were not affected by treatments.

Winter season emerged weed flora

Forage allowance and stocking method affected the density of emerged weed flora in the winter grazing season (P<0.001 and 0.01, respectively), but the summer crop rotation did not affect the emerged weed flora density in the grazing season (P>0.05). Significant interactive effects between forage allowance and stocking method (P<0.05) indicated that continuous stocking with moderate forage allowance resulted in greater emerged weed flora than did the other treatments, whereas the emerged weed flora density under rotational stocking did not differ between high and moderate forage allowance (Fig. 2). *Digitaria horizontalis* and *S. media* plant densities significantly increased (P<0.05) under continuous stocking but not under rotational stocking (Table 3).

The weed species that emerged in the winter grazing season and that were significantly affected by forage allowance are shown in Table 3. For the majority of the weed species, high forage allowance resulted in lower emergence than did moderate forage allowance. However, the emergence of *A. lividus* showed the opposite effect of forage allowance.

Summer season emerged weed flora

During the summer (crop) phase, significant differences in total emerged weed flora densities were found only between forage allowance treatments (P<0.01; Fig. 3). The emerged weed flora density was lower under high forage allowance than under moderate forage allowance. The species whose emerged weed flora densities were higher under high forage allowance than under moderate forage allowance included *Malvastrum coromandelianum*, *Richardia brasiliensis* and *Conyza bonariensis* (Table 4).

At the harvest phase of the summer season, the emerged weed flora density was significantly affected by all treatments (forage allowance, P<0.001; stocking method, P<0.001; and crop rotation, P<0.04), and forage allowance exhibited significant interactive effects with stocking method and crop rotation (P<0.01; Fig. 4). Compared with moderate forage allowance, high forage allowance resulted in a lower emerged weed flora density. In addition, the moderate forage allowance resulted in higher emerged weed flora densities under rotational stocking than under continuous stocking, as well as under a maize-soybean summer crop rotation than under a soybean monoculture. The species affected by each treatment are shown in Table 5.

Weed community composition and structure in an ICLS

A total of 30 weed species representing 16 families (25 dicotyledonous and 5 monocotyledonous as well as 19 annuals and 11 perennials) were identified in the soil seed bank and in the emerged weed flora (Table 1). *Amaranthus lividus* L., *C. brevifolius* (Rottb.) Hassk., and *Stellaria media* (L.) Vill. dominated the soil seed bank and together accounted for 74.6% of total. The dominant emerged weed flora included *A. lividus* during the winter and summer in the crop phase (45.9% and 47.4% of the emerged weed flora, respectively), and *S. media* was dominant during the summer in the harvest phase and comprised 82.3% of the total emerged weed flora (Table 2).

The results of the NMDS (Fig. 5) showed that a shift in the species composition of both the weed seed bank and emerged weed flora (winter+summer emerged weed flora) occurred in response to only the forage allowance factor via the dissimilarity matrix that was subjected to the PerMANOVA test ($P<0.001$). No differences were found by the PerMANOVA test of the Bray-Curtis dissimilarity matrix regarding the weed seed bank and emerged weed flora species composition for crop rotation or stocking method ($P>0.70$).

The analysis of the community structure revealed that crop rotation and grazing management (forage allowance and stocking method) did not affect species richness, the Shannon diversity index, or evenness in the weed seed bank ($P>0.05$). For the emerged weed flora (winter+summer), forage allowance significantly affected the species richness (Table 6) and evenness of the emerged weed flora (Table 6). Moderate forage allowance resulted in higher species richness and lower evenness than did high forage allowance. Overall, the low evenness values indicate that the species community was dominated by a single species (Hosseini *et al.* 2014). Compared with that under rotational stocking, the emerged weed flora under continuous stocking had a significantly higher Shannon diversity index value (Table 6), indicating a more diverse weed community.

Discussion

Studies conducted in other regions of the world have demonstrated that integrating livestock grazing into diversified crop-based systems can reduce weed infestations in arable lands (Tracy & Davis 2009; Miller *et al.* 2015; Lehnhoff *et al.* 2017). In the present study, we have demonstrated that the magnitude of weed infestation in an ICLS depends on grazing management (i.e., forage allowance and stocking method) and its interaction with crop rotation. Furthermore, the current research was performed under no-tillage management, whereas other studies have been conducted in tilled management systems (Tracy & Davis

2009; Miller *et al.* 2015). The current results also provide new insight with robust evidence that forage allowance, along with the standard management of no-tillage ICLS (i.e., crop rotation and grazing management), is the major factor that determines weed outcomes. Competition for light, forage regrowth, and both defoliation and possible species selection by grazing animals are direct impacts that can occur during the grazing phase in winter, whereas residue cover during the summer row-cropping phase likely results in a physical barrier to weed emergence. All of these impacts may potentially be affected by forage allowance.

Changes in the weed seed bank in response to crop rotation and grazing management in an ICLS

The soil seed bank is the primary source of weed infestation in agricultural fields, and management practices that reduce weed seed banks are valuable for integrated weed control methods (Davis *et al.* 2006). The results of the present study indicate that high forage allowance in an ICLS under no-tillage management with a sheep-grazed winter cover crop significantly reduced the weed seed bank size and specifically reduced the number of seeds of two particularly problematic species: *C. bonariensis* and *S. media*. These weeds produce seed during harvest season, as incomplete control by herbicides and moderate forage allowance allowed relatively more plants of these species to produce seed each year. These results agree with those of Schuster *et al.* (2016), who reported that reducing the grazing intensity of cattle (i.e., increasing the forage allowance) under no-tillage management reduced the seed bank size in an ICLS. Therefore, high forage allowance may be required to reduce the weed seed bank in an ICLS under no-tillage management independently of the nature of the grazing species, as the same response has now been observed between sheep and cattle grazing. It is possible that the high forage allowance led to greater competitive pressure on these species,

reducing their development and fecundity and, as a consequence, reducing their seed bank size and recruitment.

In the current study, more than 70% of the weed seed bank was found in the 0-5-cm soil layer, and we found no treatment effects on the seed bank size at the 5-10-cm soil depth. Under no-tillage management, weed seeds accumulate primarily in the top layer of the soil (Cardina *et al.* 2002), although some seeds can infiltrate soil over time via cracks, transport by fauna, and thermohydric cycles (Dorado *et al.* 1999). The results of the present study indicate that the treatments did not largely influence the factors that drive the vertical distribution of seeds in the no-tillage ICLS despite the treatments having been in place for a 12-year period.

Changes in emerged weed flora in response to crop rotation and grazing management in an ICLS

Winter-grazed cover crop phase

Higher forage allowance maintained more crop biomass and higher vegetative cover than did moderate forage allowance. The higher vegetative cover exerted greater competition, resulting in the suppression of many weeds during the winter pasture phase of the ICLS. Thus, high forage allowance could potentially be used as a management tool in an ICLS. However, *A. lividus* responded oppositely to forage allowance (i.e., lower emerged weed flora density in the moderate forage allowance treatment than under high forage allowance), probably because plants of this genus are grazed selectively by sheep (Ramos *et al.* 2005). Decreased forage allowance reduces animal feed selection, and this reduction could result in increased intake of this species by sheep under moderate forage allowance. Furthermore, annual dicotyledonous plants in general are more vulnerable to frequent defoliation than are perennials and grasses (Meiss *et al.* 2008). Moreover, De Bruijn & Bork (2006) reported that rotational stocking

depends on high grazing intensities for effective weed control in perennial pastures. In accordance, the current results demonstrated that weed suppression by increased grazing intensity (i.e., the use of moderate forage allowance instead of high forage allowance) was greater under rotational management than under continuous stocking management, and specifically, rotational stocking reduced the number of plants of *D. horizontalis* and *S. media*. Compared with continuous stocking, rotational stocking can also reduce animal feed selection, resulting in the intake of these species.

The current findings revealed that, during the winter-grazed cover crop phase, two distinct methods occur for weed suppression, each with different trade-offs. First, relatively low grazing intensity (high forage allowance) results in more cover crop biomass and increased weed suppression by competitive exclusion for some weeds; however, these actions reduce the system efficiency because less grazing can result in reduced gains or increased need for supplemental feed for animals, reducing overall profits from livestock production (Neto *et al.* 2014). Second, relatively high grazing intensity (moderate forage allowance) and the use of rotational stocking potentially reduces animal feed selection, which could result in increased weed intake and a subsequent reduction in the density of some weeds. However, increased grazing intensity can reduce overall residual biomass, which can reduce soil cover and thus weed suppression during summer row cropping (Schuster *et al.* 2016).

Summer crop phase

Winter forage allowance for grazing animals determines the amount of pasture residue that remains when summer row crops are seeded in an ICLS under no-tillage management (Schuster *et al.* 2016). A high forage allowance increases residual straw (Kunrath *et al.* 2014), which provides a physical barrier that can reduce emerged weed flora (Webster *et al.* 2016), corroborating with current results. For example, the current results showed lower *C.*

bonariensis emergence under high forage allowance than under moderate forage allowance (Table 3). Wu *et al.* (2007) reported that *C. bonariensis* seeds are positively photoblastic, so increased pasture residues that better cover the soil during the summer crop phase could sufficiently restrict light penetration into the soil during a fallow situation to reduce *C. bonariensis* germination.

After crop harvest, producers often allow weeds to grow uncontrolled, but controlling these weeds is important to avert new seed deposits into the seed bank, which is the main source of future weed infestations in arable lands. In the present study, high forage allowance reduced weed population densities after harvest in the ICLS, possibly because residual straw had the same suppressive effects as those reported above during the crop growing season. Moreover, several studies have demonstrated that, compared with crop monocultures, crop rotations reduce weed populations (Liebman & Dyck 1993). Nevertheless, the current study showed contrasting results in the harvest phase; weed populations in the soybean-maize rotation were higher than those in the soybean monoculture. However, this effect was closely linked to the effects of significant increases in seedling emergence of specific weeds (i.e., *S. media*) in the crop rotation treatments *S. media* is among the 12 most successful opportunistic species to colonize fields (Holm *et al.* 1991). This species can grow and reproduce in a very short time and is adapted to a wide range of habitats; these attributes partially explain why this species was most abundant among the emerged weed in the current study. Additional research is needed to determine how management factors in ICLSs, especially summer crop rotations and stocking method under moderate forage allowance, affect *S. media* in an ICLS.

Weed community composition and structure in an ICLS subjected to long-term crop rotation and grazing management

The current ICLS results with a winter-grazed grass cover crop under no-tillage management demonstrated that forage allowance is a more important long-term ecological filter for determining weed outcomes than are stocking method and summer crop rotation. In agricultural landscapes, weeds behave as a metacommunity, and different types of management practices act as filters (i.e., preventing or facilitating weed invasion or seed bank expression) that ultimately affect the weed community at the field level. Species richness in the seed bank was not affected by forage allowance and was higher in the emerged weed flora under moderate forage allowance than under high forage allowance. This finding provides evidence that high forage allowance filters the weed seed bank expression better than a moderate forage allowance both in terms of the density of emerged weed flora as well as the number of species present. Increased similarity between the weed seed banks and emerged weed flora resulting from reduced forage allowance was previously reported by Schuster *et al.* (2016) in a similar ICLS experiment.

In the past century, weed diversity has drastically decreased in arable lands (Robinson & Sutherland 2002), and several studies have drawn attention to the need to conserve weed diversity in croplands (Gaba *et al.* 2016). The importance of weed diversity can be viewed as a trade-off between (1) the benefits of providing ecosystem services (e.g., trophic offerings for herbivores and pollinators) and (2) the harmful effects on crop production (e.g., alternate hosts for pathogens and insect pests, crop-weed competition losses, and harvesting difficulties) (Mézière *et al.* 2015). The current results showed that continuous stocking in the winter-grazed grass cover crop under no-tillage management promoted greater weed diversity than did rotational stocking at the plot scale (Table 6), which is adequate for evaluating how biotic factors can affect species community structure (Perronne *et al.* 2017). However, for a functional analysis of both ecosystem services and the harmful effects of weeds, it is necessary to use data from a regional scale to assess the entire species pool. Other

environmental advantages of continuous stocking have been reported previously. For example, Savian *et al.* (2014) demonstrated that, compared with rotational stocking, continuous stocking reduced methane emissions per unit animal.

Limits of the present results

Production systems that have increasingly diverse and dynamic components, such as ICLSs, create complexity that shifts the living plant and soil communities further along the continuum of spatial-temporal grassland-cropping succession. In the present study, the ICLS was characterized by bi-annual no-tillage crop-pasture rotation; these characteristics differ from those of other ICLS types used in temperate regions, which are mostly based on multiannual tillage pasture-crop rotations. Under the latter, the impact of crop-pasture management on weed dynamics could differ from that resulting from the current data. Moreover, due to the characteristics of the present investigation, the dataset tends to produce a descriptive picture of an ICLS under a specific pedoclimate and weed species pool conditions. Therefore, these findings are not applicable to all production systems particularly because the current results showed that different weed species reacted differently to grazing management. Thus, for a deep, mechanistic understanding of the reasons for effective weed control in ICLSs, future studies are needed to disentangle animal-weed interactions and identify weed traits and livestock grazing management practices that can be mutually beneficial for weed control, livestock grazing management and the environment. Modeling approaches have been used to provide substantial insight into the interactions between weeds, management practices, and the environment (Colbach *et al.* 2014), and these models could represent means for achieving a deeper understanding of weed dynamics in an ICLS.

Summary and Concluding Remarks

This study adds to the existing knowledge of cropping systems-weed interactions demonstrating that the effects of crop rotation or grazing management on weeds in an ICLS with a winter-grazed grass cover crop under no-tillage management, with or without interactive effects between them, depended on the weed species pool and the time at which those weed species emerged. Furthermore, the data also indicate that, in this type of ICLS, whole-system weed management benefits from maintaining a high forage allowance during the grazing phase. In addition, the data specifically showed that high forage allowance neutralizes negative weed outcomes (i.e., increased total emerged weed flora density) that occurred in conjunction with moderate forage allowance both from continuous stocking during the winter grazing season and from crop rotation and rotational stocking during the summer in the harvest phase. Therefore, managers should generally account for the occurrence of complex assemblies of weeds in the agricultural landscape, and farms should adopt high forage allowances to reduce emerged weed flora density and seed banks in this type of ICLS. However, if managers want to prioritize livestock production via increased grazing intensity (i.e., lower forage allowance), they should use alternatives management practices that avoid the trade-off between livestock efficiency and residual biomass that covers the soil during the row crop phase, such as delaying winter-grazed cover crop desiccation after the grazing period to allow more cover crop residue.

References

Bellinder, R. R., Dillard, H. R. and Shah, D. A. (2004). Weed seedbank community responses to crop rotation schemes. *Crop Protection* **23**, 95–101.

Bray, J. R. and Curtis, J. T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs* **27**, 325–349.

Busi, R., Vila-Aiub, M. M., Beckie, H. J., Gaines, T. A., Goggin, D. E., Kaundun, S. S., Lacoste, M., Neve, P., Nissen, S. J., Norsworthy, J. K., Renton, M., Shaner, D. L., Tranel, P. J., Wright, T., Yu, Q. and Powles, S. B. (2013). Herbicide-resistant weeds: from research and knowledge to future needs. *Evolutionary Applications* **6**, 1218–1221.

Cardina, J., Herms, C. P. and Doohan, D. J. (2002). Crop rotation and tillage system effects on weed seedbanks. *Weed Science* **50**, 448–460.

Cauwer, B., van den Berge, K., Cougnon, M., Bulcke, R. and Reheul, D. (2010). Weed seed bank responses to 12 years of applications of composts, animals urines or mineral fertilizers. *Weed Research* **50**, 425–435.

Colbach, N., Biju-Duval, L., Gardarin, A., Granger, S., Guyot, S. H., Meziere, D., Munier-Jolain, N. and Petit, S. (2014). The role of models for multicriteria evaluation and multiobjective design of cropping systems for managing weeds. *Weed Research* **54**, 541–555.

Davis, A. S., Anderson, K. I., Hallett, S. G. and Renner, K. A. (2006). Weed seed mortality in soils with contrasting agricultural management histories. *Weed Science* **54**, 291–297.

De Bruijn, S. L. and Bork, E. W. (2006). Biological control of *Canada thistle* in temperate pastures using high density rotational cattle grazing. *Biological Control* **36**, 305–315.

De Moraes, A., Carvalho, P. C. F., Anghinoni, I., Lustosa, S. B. C., Costa, S. V. E. G. A., Kunrath, T. R. (2014). Integrated crop–livestock systems in the Brazilian subtropics. *European Journal of Agronomy* **57**, 4–9.

Dorado, J., Monte, J. and Lopez-Fando, C. (1999). Weed seedbank response to crop rotation and tillage in semiarid agroecosystems. *Weed Science* **47**, 67–73.

Fedoroff, N. V., Battisti, D. S., Beachy, R. N., Cooper, P. J. M., Fischhoff, D. A., Hodges, C. N., Knauf, V. C., Lobell, D., Mazur, B. J., Molden, D., Reynolds, M. P., Ronald,

P. C., Rosegrant, M. W., Sanchez, P. A., Vonshak, A. and Zhu, J. (2010). Radically rethinking agriculture for the 21st century. *Science* **327**, 833–834.

Gaba, S., Reboud, X. and Fried, G. (2016). Agroecology and conservation of weed diversity in agricultural lands. *Botany Letters* **163**, 351–354.

Herrero, M., Thornton, P. K., Notenbaert, A. M., Wood, S., Msangi, S., Freeman, H. A., Bossio, D., Dixon, J., Peters, M., Van De Steeg, J., Lynam, J., Parthasarathy Rao, P., Macmillan, S., Gerard, B., McDermott, J., Sere, C. and Rosegrant, M. (2010). Smart investments in sustainable food production: revisiting mixed crop-livestock systems. *Science* **327**, 822–825.

Holm, L. R. G., Pancho, J., Herberger, J. and Plucknett, D. (1991). *A Geographical Atlas of World Weeds*. Malabar: Krieger Publishing Company.

Hosseini, P., Karimi, H., Babaei, S., Mashhadi, H. R. and Oveisi, M. (2014). Weed seed bank as affected by crop rotation and disturbance. *Crop Protection* **64**, 1–6.

Kissmann, K. G. and Groth, D. (1997). *Plantas Infestantes e Nocivas*. São Paulo: BASF.

Kunrath, T. R., Cadenazzi, M., Brambilla, D. M., Anghinoni, I., De Moraes, A., Barro, R. S. and De Carvalho, P. C. F. (2014). Management targets for continuously stocked mixed oat×annual ryegrass pasture in a no-till integrated crop–livestock system. *European Journal of Agronomy* **57**, 71–76.

Lacey, J. R. and Sheley, R. L. (1996). Leafy spurge and grass response to picloram and intensive grazing. *Journal of Range Management* **49**, 311–314.

Lechenet, M., Deytieux, V., Antichi, D., Aubertot, J.-N., Bàrberi, P., Bertrand, M., Cellier, V., Charles, R., Colnenne-David, C., Dachbrodt-Saaydeh, S., Debaeke, P., Doré, T., Farcy, P., Fernandez-Quintanilla, C., Grandeau, G., Hawes, C., Jouy, L., Justes, E., Kierzek, R., Kudsk, P., Lamichhane, J. R., Lescourret, F., Mazzoncini, M., Melander, B., Messéan, A., Moonen, A.-C., Newton, A. C., Nolot, J.-M.,

Panozzo, S., Retaureau, P., Sattin, M., Schwarz, J., Toqué, C., Vasileiadis, V. P. and Munier-Jolain, N. (2017). Diversity of methodologies to experiment integrated pest management in arable cropping systems: analysis and reflections based on a European network. *European Journal of Agronomy* **83**, 86–99.

Lehnhoff, E., Miller, Z., Miller, P., Johnson, S., Scott, T., Hatfield, P. and Menalled, D. F. (2017). Organic agriculture and the quest for the Holy Grail in water-limited ecosystems: managing weeds and reducing tillage intensity. *Agriculture* **7**, 4–8.

Lemaire, G., Franzluebbers, A., De Carvalho, P. C. F. and Dedieu, B. (2014). Integrated crop–livestock systems: strategies to achieve synergy between agricultural production and environmental quality. *Agriculture, Ecosystems & Environment* **190**, 4–8.

Liebman, M. and Dyck, E. (1993). Crop rotation and intercropping strategies for weed management. *Ecological Applications* **3**, 92–122.

Lorenzi, H. (2006). *Manual de Identificação e Controle de Plantas Daninhas*. São Paulo: Instituto Plantarum de Estudos da Flora.

Lustosa, S. B. C., Schuster, M. Z., Martinichen, D., Pelissari, A. and Gazziero, D. L. P. (2016). Floristic and phytosociology of weed in response to winter pasture sward height at integrated crop-livestock in Southern Brazil. *Brazilian Journal of Applied Technology for Agricultural Science* **9**, 19–26.

McCune, B., Grace, J. B. and Urban, D. L. (2002). *Analysis of Ecological Communities*. Gleneden Beach: MjM Software Design.

McKenzie, S. C., Goosey, H. B., O'Neill, K. M. and Menalled, F. D. (2016). Impact of integrated sheep grazing for cover crop termination on weed and ground beetle (*Coleoptera*: *Carabidae*) communities. *Agriculture, Ecosystems & Environment* **218**, 141–149.

Meiss, H., Munier-Jolain, N., Henriot, F. and Caneill, J. (2008). Effects of biomass, age and functional traits on regrowth of arable weeds after cutting. *Journal of Plant Diseases and Protection* **21**, 493–499.

Mézière, D., Colbach, N., Dessaint, F. and Granger, S. (2015). Which cropping systems to reconcile weed-related biodiversity and crop production in arable crops? An approach with simulation-based indicators. *European Journal of Agronomy* **68**, 22–37.

Miller, Z. J., Menalled, F. D., Sainju, U. M., Lenssen, A. W. and Hatfield, P. G. (2015). Integrating sheep grazing into cereal-based crop rotations: spring wheat yields and weed communities. *Agronomy Journal* **107**, 104–112.

National Research Council [NRC] (1985). *Nutrient Requirements of Sheep*. Washington, DC: National Academy of Science Press.

Neto, A. B., Savian, J. V., Schons, R. M. T., Bonnet, O. J. F., Do Canto, M. W., De Moraes, A., Lemaire, G. and De Carvalho, P. C. F. (2014). Italian ryegrass establishment by self-seeding in integrated crop-livestock systems: effects of grazing management and crop rotation strategies. *European Journal of Agronomy* **53**, 67–73.

Nie, Z., McLean, T., Clough, A., Tocker, J., Christy, B., Harris, R., Riffkin, P., Clark, S. and McCaskill, M. (2016). Benefits, challenges and opportunities of integrated crop-livestock systems and their potential application in the high rainfall zone of southern Australia: a review. *Agriculture, Ecosystems & Environment* **235**, 17–31.

Onofri, A., Carbonell, E. A., Piepho, H. P., Mortimer, A. M. and Cousens, R. D. (2010). Current statistical issues inWeed Research. *Weed Research* **50**, 5–24.

Perronne, R., Munoz, F., Borgy, B., Reboud, X. and Gaba, S. (2017). How to design trait-based analyses of community assembly mechanisms: insights and guidelines from a literature review. *Perspectives in Plant Ecology, Evolution and Systematics* **25**, 29–44.

Pontes, L. S., Nabinger, C., De Carvalho, P. C. F., Da Trindade, J. K., Montardo, D. P. and Dos Santos, R. J. (2003). Variáveis morfogênicas e estruturais de azevém anual (*Lolium multiflorum* Lam.) manejado em diferentes alturas. *Revista Brasileira de Zootecnia* **32**, 814–820.

Ramos, J. J., Ferrer, L. M., García, L., Fernández, A. and Loste, A. (2005). Polioencephalomalacia in adult sheep grazing pastures with prostrate pigweed. *The Canadian Veterinary Journal* **46**, 59–61.

Renne, I. J. and Tracy, B. F. (2013). Disturbance intensity, timing and history interact to affect pasture weed invasion. *Basic and Applied Ecology* **14**, 44–53.

Robertson, G. P. and Swinton, S. M. (2005). Reconciling agricultural productivity and environmental integrity: a grand challenge for agriculture. *Frontiers in Ecology and the Environment* **3**, 38–46.

Robinson, R. A. and Sutherland, W. J. (2002). Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology* **39**, 157–176.

Savian, J. V., Neto, A. B., De David, D. B., Bremm, C., Schons, R. M. T., Genro, T. C. M., Do Amaral, G. A., Gere, J., McManus, C. M., Bayer, C. and de Faccio Carvalho, P. C. (2014). Grazing intensity and stocking methods on animal production and methane emission by grazing sheep: implications for integrated crop–livestock system. *Agriculture, Ecosystems & Environment* **190**, 112–119.

Schuster, M. Z., Pelissari, A., De Moraes, A., Harrison, S. K., Sulc, R. M., Lustosa, S. B. C., Anghinoni, I. and Carvalho, P. C. F. (2016). Grazing intensities affect weed seedling emergence and the seed bank in an integrated crop–livestock system. *Agriculture, Ecosystems & Environment* **232**, 232–239.

Sulc, R. M. and Franzluebbers, A. J. (2014). Exploring integrated crop–livestock systems in different ecoregions of the United States. *European Journal of Agronomy* **57**, 21–30.

Thompson, K., Bakker, J. P. and Bekker, R. M. (1997). *The soil seed banks of North West Europe: Methodology, Density and Longevity*. Cambridge, UK: Cambridge University Press.

Tracy, B. F. and Davis, A. S. (2009). Weed biomass and species composition as affected by an integrated crop–livestock system. *Crop Science* **49**, 1523–1530.

United States Department of Agriculture [USDA] (1999). *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*. Washington DC, USA,: Natural Resources Conservation Service, USDA.

Webster, T. M., Simmons, D. B., Culpepper, A. S., Grey, T. L., Bridges, D. C. and Scully, B. T. (2016). Factors affecting potential for *Palmer amaranth* (*Amaranthus palmeri*) suppression by winter rye in Georgia, USA. *Field Crops Research* **192**, 103–109.

Wu, H., Walker, S., Rollin, M. J., Tan, D. K. Y., Robinson, G. and Werth, J. (2007). Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* [L.] Cronquist). *Weed Biology and Management* **7**, 192–199.

Figure legends

Fig. 1. Effects of forage allowance on changes in the total weed seed bank size (seeds/m^2) in the 0-10-cm, 0-5-cm- and 5-10-cm soil layers after 12 years in an integrated crop-livestock system in southern Brazil. Asterisks (*) denote a significant difference between grazing intensities at α=0.05; NS=not significant according to a mixed-model ANOVA. The vertical error bars represent the standard deviations

Fig. 2. Effects of forage allowance and stocking method on emerged weed flora density (plants/m^2) in the winter-grazed cover crop of an integrated crop-livestock system in southern Brazil. The means with different letters indicate significant differences according to the Tukey HSD test ($P<0.05$). The vertical error bars represent the standard deviations. CS: continuous stocking; RS: rotational stocking

Fig. 3. Effect of forage allowance on emerged weed flora density (plants/m^2) in the summer season during the cropping phase of an integrated crop-livestock system in southern Brazil. The means were significantly different ($P<0.05$) according to a mixed-model ANOVA. The vertical error bars represent the standard deviations

Fig. 4. Effects of stocking method, crop rotation, and forage allowance on emerged weed flora density (plants/m^2) in the summer season at the crop harvest phase of an integrated crop-livestock system in southern Brazil. The means with different letters indicate significant differences according to the Tukey HSD test ($P<0.05$). The vertical error bars represent the standard deviations. CS: continuous stocking; RS: rotational stocking; S-S: soybean-soybean; S-M: soybean-maize rotation

Fig. 5. Non-metric multidimensional scaling ordination plots based on the species composition of emerged weed flora (winter+summer) and the seed bank in an integrated crop-livestock system in southern Brazil. The stress value was 0.1127. The ordinations are based on the Bray-Curtis dissimilarity matrix for relative abundance. The circle symbols represent

emerged weed flora (winter+summer), and the squares represent the seed bank; the filled symbols represent high forage allowance, and the open symbols represent moderate forage allowance

Tables

Table 1. Average density of seed bank and emerged flora (seeds at a 0-10-cm depth or plants/m^2) of weed species present in an integrated crop-livestock system in southern Brazil

Species	Family	Functional group[1]	Seed banks[2]	Emerged weed flora		
				Winter season	Summer season	
					Crop phase	Harvest phase
Amaranthus lividus L.	Amaranthaceae	ADG	1173.7	8.5	18.8	1.7
Bidens pilosa L.	Asteraceae	ADU		0.7	0.5	0.1
Bowlesia incana Ruiz & Pav.	Apiaceae	ADU	2.6	0.5		0.1
Commelina bengalensis L.	Commelinaceae	PDU			0.1	
Commelina nudiflora L.	Commelinaceae	PDU			0.1	
Conyza bonariensis L. Cronquist	Asteraceae	ADU	192.4	0.1	3.4	6.2
Coronopus didymus (L.) Sm.	Brassicaceae	ADO	211.9		0.5	0.1
Cyperus brevifolius (Rottb.) Hassk.	Cyperaceae	AMO	1018.2	0.1	1.4	0.1
Digitaria horizontalis Willd.	Poaceae	AMO		0.9	0.3	0.1
Eleusine indica (L.) Gaertn.	Poaceae	AMO	195.4	0.9	1.2	0.2
Eragrostis plana Ness.	Poaceae	PMO		0.1		
Euphorbia heterophylla L.	Euphorbiaceae	ADU			0.1	
Facelis retusa (Lam.) Schultz-Bip.	Asteraceae	ADU	9.6	0.4		0.5
Galinsoga quadriradiata Ruiz & Pav.	Asteraceae	ADU	37.4			0.1
Gnaphalium spicatum Lam.	Compositae	ADU	82.5	0.9	0.1	2.0
Hydrocotyle bonariensis Lam.	Araliaceae	PDU	18.2	1.3	0.1	0.1
Lepidium pseudodidymum Thell.	Brassicaceae	ADU				0.8
Malvastrum coromandelianum (L.) Garcke	Malvaceae	PDU	476.9	0.3	1.7	1.5
Oxalis corniculata L.	Oxalidaceae	PDO		0.1		0.3
Oxalis latifolia L.	Oxalidaceae	PDO		0.1		0.2
Portulaca oleracea L.	Portulacaceae	ADG	68.6		0.6	
Richardia brasiliensis Gomes	Rubiaceae	PDU	57.3	0.1	1.9	1.6
Senecio brasiliensis Less.	Asteraceae	PDU	10.4		0.2	0.1
Silene gallica L.	Caryophyllaceae	ADU				0.1
Solanum americanum Mill.	Solanaceae	PDU	52.1	0.1	0.1	0.1
Soliva pterosperma (Juss.) Less	Asteraceae	ADU	1.7		0.1	0.2
Sonchus oleraceus L.	Asteraceae	ADO	68.6			0.1
Stachys arvensis L.	Lamiaceae	ADU		0.1	0.4	
Stellaria media (L.) Vill.	Caryophyllaceae	ADO	3136.0	2.5		81.0
Urochloa plantaginea (Link) Hitchc.	Poaceae	AMG	320.6	0.9	8.0	1.1

[1]A: annual; P: perennial; D: dicotyledonous; M: monocotyledonous; U: unpalatable by sheep; G: grazed by sheep; O: occasionally grazed by sheep. [2]Blank fields indicate that no species were found.

Table 2. Forage allowance and summer crop rotation effects on the species-level weed seed banks (seeds/m^2 at a depth of 0-5 cm) of an integrated crop-livestock system in southern Brazil

Species	Forage allowance	
	High	Moderate
Conyza bonariensis (L.) Cronquist**	38.1	346.6
Stellaria media (L.) Vill.**	534.6	5738.4

** represent significant effects at P≤0.05 according to ANOVA.

Table 3. Effects of forage allowance and stocking method on emerged weed flora (plants/m^2) in the winter season (grazing phase) of an integrated crop-livestock system in southern Brazil

Species	Forage allowance	
	High	Moderate
Amaranthus lividus L.**	11.3	5.8
Bidens pilosa L.**	0.2	1.1
Eleusine indica (L.) Gaertn**	0.1	1.8
Facelis retusa (Lam.) Schultz-Bip**	0.1	0.8
Gnaphalium spicatum Lam.**	0.3	1.6
Hydrocotyle bonariensis Lam.**	0.4	2.2
Stellaria media (L.) Vill.*	1.4	3.6
	Stocking method	
	Continuous	Rotational
Digitaria horizontalis Willd.*	2.7	0.4
Stellaria media (L.) Vill.*	3.7	1.2

* and ** represent, respectively, significant effects at $P \leq 0.05$ and $P \leq 0.01$ according to ANOVA.

Table 4. Effects of forage allowance, stocking method and crop rotation on emerged weed flora (plants/m^2) in the summer season (crop growth phase) of an integrated crop-livestock system in southern Brazil

Species	Forage allowance	
	High	Moderate
Conyza bonariensis (L.) Cronquist**	1.1	5.6
Malvastrum coromandelianum (L.) Garcke*	0.6	2.7
Richardia brasiliensis Gomes*	0.1	3.6
	Crop rotation	
	Soyabean-Soyabean	Soyabean-Maize
Urochloa plantaginea (Link) Hitchc.**	6.0	10.1

* and ** represent, respectively, significant effects at $P \leq 0.05$ and $P \leq 0.01$ according to ANOVA.

Table 5. Effects of forage allowance, stocking method and crop rotation on emerged weed flora (plants/m^2) in the summer season (crop harvest phase) of an integrated crop-livestock system in southern Brazil

Species	Forage allowance	
	High	Moderate
Conyza bonariensis (L.) Cronquist*	3.6	8.8
Malvastrum coromandelianum (L.) Garcke*	0.5	2.6
Richardia brasiliensis Gomes*	0.2	3.1
Stellaria media (L.) Vill.**	5.3	156.8
	Stocking method	
	Continuous	Rotational
Gnaphalium spicatum Lam.*	5.6	1.8
Stellaria media (L.) Vill.**	39.7	122.4
	Crop rotation	
	Soyabean-Soyabean	Soyabean-Maize
Stellaria media (L.) Vill.*	58.9	103.1

* and ** represent, respectively, significant effects at $P \leq 0.05$ and $P \leq 0.01$ according to ANOVA.

Table 6. Effects of forage allowance and stocking method on weed species richness (number of species), Shannon diversity index and evenness of an integrated crop-livestock system in southern Brazil

	Forage allowance	
	High	Moderate
Species richness**	17	23
Evenness**	0.44	0.33
	Stocking method	
	Continuous	Rotational
Shannon diversity**	2.12	1.83

**Significant at P≤0.01 according to a mixed-model ANOVA.

Figures

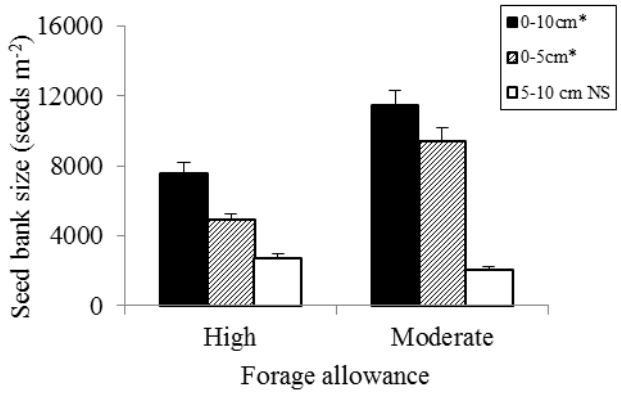
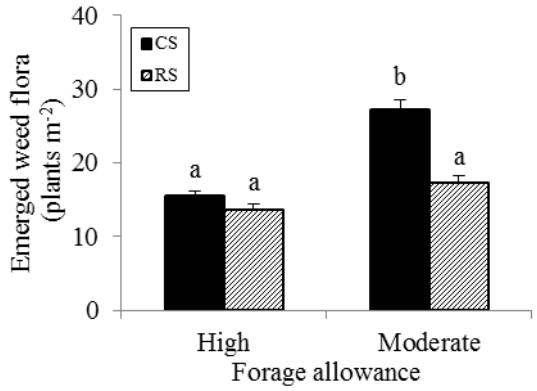


Fig. 1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CS
RS
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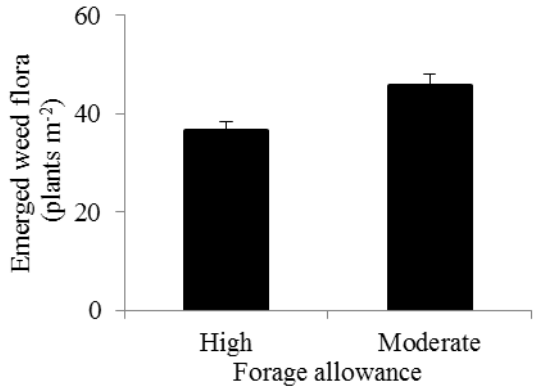
Emerged weed flora (plants m-2)
High
Moderate
Forage allowance

Fig. 2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40
20
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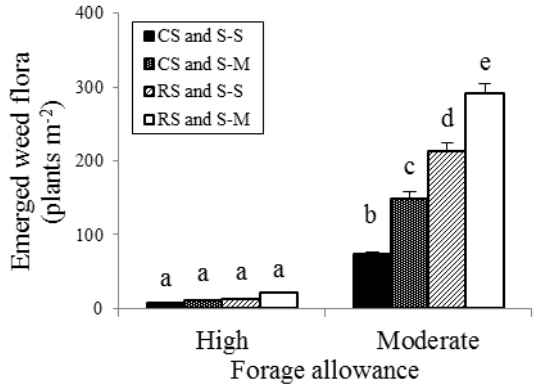
Emerged weed flora (plants m-2)
High
Moderate
Forage allowance

Fig. 3


400
300
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100
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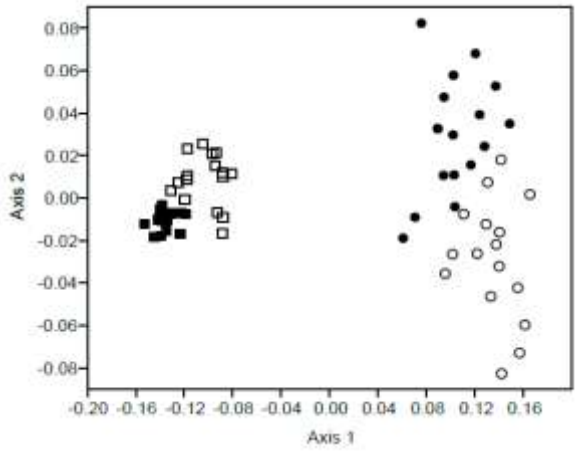
Emerged weed flora (plants m-2)
CS and S-S
CS and S-M
RS and S-S
RS and S-M
a a a a
b
c
d
e
High
Moderate
Forage allowance

Fig. 4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0.06
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Axis 2
-0.20 -0.16 -0.12 -0.08 -0.04 0.00 0.04 0.08 0.12 0.16
Axis 1

Fig. 5

CHAPTER 3

Simple rule for lower glyphosate input and greater soybean and beef output on integrated crop-livestock systems[1]

[1] Prepared in accordance with the standards of the *Agricultural Systems*.

Simple rule for lower glyphosate input and greater soybean and beef output on integrated crop-livestock systems

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Abstract

Pesticide inputs for food and feed production is a global concern regarding food security, human health, and environmental quality. Many current agricultural landscapes would benefit by undergoing an agroecological redesign in order to reduce pesticide inputs and improve sustainability of farming systems. This study aimed to evaluate how forage allowances (based on differing pasture sward height) in integrated crop-livestock systems (ICLS) affect the need for glyphosate use, as well as soybean (*Glycine max* (L.) Merrill) and beef output in a succession of mixed black oat (*Avena strigosa* Schreb.) + Italian ryegrass (*Lolium multiflorum* Lam.) cover crop grazed by cattle during the winter followed by soybean production during the summer. We hypothesized that there is an optimal sward height range during the grazing period that would maximize soybean and beef production while minimizing glyphosate use in ICLS. The experiment was conducted in several commercial fields involving two experimental protocols (one short-term and one long-term experiment) in Southern Brazil. Our results indicated that using moderate forage allowance in cover crops (i.e., maintaining 17.8 cm of pasture sward height) allowed reduced glyphosate use and increased soybean and beef production in ICLS, when compared with lower forage allowance (i.e. <10.7 cm of pasture sward height). High forage allowance (i.e. >17.8 cm sward height) necessitated lower glyphosate doses but resulted in lower beef production. Therefore, moderate forage allowance can promote greater soybean and beef production with lower glyphosate inputs in ICLS.

Keywords: Sustainable glyphosate use; integrated weed management; crop yield; weed ecology; landscape ecology.

1. Introduction

A major technological change in agriculture beginning in the 20th century was the widespread use of herbicides for weed control. Nowadays, most food and feed production systems rely on the use of synthetic herbicides for weed management (Boissinot et al., 2011). The widespread adoption of genetically modified glyphosate-resistant (GR) crops has promoted a dramatic increase in glyphosate use and a reduction in the use of other herbicides in cropland (Beckie, 2011). Benbrook (2016) reported that 8.6 billion kilograms of glyphosate have been sprayed globally on crops since the mid-1970s, from which approximately 265 million kilograms per year was used for soybean crops.

Glyphosate use stimulated a long-running debate about its risk to human health and it was classified in 2015 as a "probable human carcinogen" by the International Agency for Research on Cancer (IARC, 2015). Despite the "probable" public health problem, glyphosate is used by producers to control weeds on 22 to 30% of cultivated cropland across the world (Benbrook, 2016). Glyphosate use has facilitated no-tillage management, greater crop yields, and it is responsible for providing additional food for millions of people globally. However, there is a recent and general call for reducing pesticide use on agricultural landscapes that is aimed toward reducing human health risks and negative impacts to the environment (Barzman & Dachbrodt-Saaydeh, 2011).

World population is projected to increase 34 % by 2050 (FAO, 2016), generating major concerns about the future of human lifestyle and food security. A reasonable way to produce enough food and fulfill societal and environmental demands is through sustainable intensification of agricultural production. Many reports have shown that integrated crop-livestock systems (ICLS) can provide synergy between agricultural production and environmental quality (Herrero et al., 2010; Lemaire et al., 2014), and these systems are widespread around the world, comprising a total area of approximately 2.5 billion hectares (Keulen and Shiere, 2004). However, Moraes et al. (2014) reported a surprising lack of

published information on pesticide use in ICLS. Researchers need to find ways to reduce pesticide use while increasing food production (Lechenet et al., 2016), preferably in the same areas currently used for agriculture in order to avoid further deforestation of native areas.

Cover crops are usually non-marketable plants grown to cover the soil surface. These crops can reduce erosion, improve soil fertility, and reduce both weed emergence and herbicide dependence (McKenzie et al., 2016). Cover crops can play a critical role in no-tillage crop management systems, because the cover crop residues are left on the soil surface and provide a physical barrier to weed emergence. Cover crops with high dry matter production and forage potential can also provide a grazing source for livestock and thus provide an additional source of revenue for farmers and increase food production per unit area between successive grain crops (Sulc and Franzluebbers, 2014; Moraes et al., 2014; Nie et al., 2016). However, other reports indicated that some of the benefits provided by cover crops depends on the production of residues left on the surface (Teasdale and Mohler, 1993; Webster et al., 2016), and grazing of cover crops may have a direct impact on the residual straw amount (Hunt et al., 2016). Generally, greater grazing intensities in cover crops result in greater meat production per area, but smaller amounts of residual straw (Kunrath et al., 2014). However, there is a lack of information about the minimum residual straw of cover crops required to provide weed control benefits in no-tillage grain crops cultivated subsequently. In ICLS of Southern Brazil, there is a particular need to determine the minimum forage allowances in winter grazed cover crops that do not negatively affect their weed control benefits in cultivated summer crops.

We hypothesized that there is an optimal grazing intensity (represented by a specific sward height) of a cover crop that maintains high beef production while still providing enough residual straw to control weeds adequately and thus reduce the need for glyphosate in ICLS. This study aimed to evaluate how forage allowance affect winter livestock production and

summer grain yields, and to determine the impact of forage allowance on the overall need for glyphosate use in subtropical ICLS.

2. Materials and Methods

2.1 Site description

2.1.1 Field farms survey

Field surveys were conducted in a region that contains the oldest ICLS established in Southern Brazil. They are located within 300 km of the city of Guarapuava in Paraná State (25º23'36" S latitude and 51º27'19" W longitude). The area is dominated by Oxisol soils, at an altitude of 1200m, characterized by a humid temperate climate with moderate hot summers (Cfb) according to the Köeppen classification system. The average annual temperature and precipitation are 18.1ºC, and 1944 mm, respectively.

The survey area included a farmers‟ network, which was started more than 20 years ago to implement ICLS. Within this long-term network, 26 fields were selected in 2014 in order to characterize sward height management during the grazing period. Those fields had some differences in agronomic history (i.e. soil fertility, agrochemical use, cultivars and crop rotation); however, during the winter season in all fields, black oat and Italian ryegrass were grown to form a mixed winter pasture. Grazing was forage-based in a continuous stocking system with steers (crossbred Angus, Simental, Charolês and Nellore) weighing 220±40 kg. Stocking was initiated when the forage height reached 25±5 cm. In the summer season, glyphosate-resistant (GR) soybean crops were cultivated under no-tillage management. None of the 26 fields had mechanical weeding methods, and they used glyphosate only for weed

control. All fields were selected from different farms that were at least 1 km apart, and some had the same owner (farmer). The averaged field area surveyed was 600 ha, with individual field sizes ranging from 50 to 2000 ha. The grazing period usually started in earlier May.

2.1.2 Long-term ICLS protocol

The long-term ICLS experiment was conducted on a 22 ha site located on Espinilho Farm (Agropecuaria Cerro Coroado) in São Miguel das Missões, Rio Grande do Sul State, Brazil (28º56'12" S latitude and 54º20'52" W longitude), at an altitude of 465m and characterized by a humid temperate climate with hot summer (Cfa) according to the Köeppen classification system. The average annual temperature and precipitation are 19ºC, and 1850 mm, respectively. The soil is an Oxisol (Rhodic Hapludox Soil Survey Staff, 1999). The soybean–beef cattle ICLS was established in 2001, and has maintained the same crop succession: (1) mixed pasture cover crop during the winter season (cattle grazing from ~May to November) and (2) soybean for grain production during the summer season (from ~December to May).

During the winter season, black oat (*Avena strigosa* cv. Iapar 61) was planted (45 kg ha^{-1}) along with naturally reseeding Italian ryegrass (*Lolium multiflorum* "common") to form a mixed pasture system. Grazing was forage-based in a continuous grazing system with steers weighing approximately 200 kg (crossbred Angus, Hereford and Nellore) that entered the pasture system when the forage reached approximately 1.5 $ton.ha^{-1}$ of dry matter (~25 cm sward height).

Treatments consisted of different sward heights during the winter season, which were determined by pasture vegetation height. Targeted pasture vegetation heights were 10, 20, 30 and 40 cm, and these heights have been maintained since the ICLS was established. All treatments were organized in a randomized block design with three replications, with

experimental units ranging from 1.0 to 2.5 ha. Pasture heights were controlled by monitoring every 14 days using the sward stick method, which consisted of using a graduated measurement stick with a "marker" that slides up and down until the first forage leaf blade is touched Approximately 100 randomized forage height measurements were taken within each experimental unit. A target average forage height, which corresponded to the desired grazing intensity treatment, was achieved using a put-and-take stocking technique (Mott and Lucas, 1952), adding or removing steers from the plot as required.

At the end of the winter season (mid-November), the area was desiccated using glyphosate (Application rates varied among fields and are described in the Results section). In December of each year (summer season), soybean was planted in rows spaced 45 cm apart using a seeding density of 45 seeds m^{-2}. Post-emergent glyphosate was applied in mid-January. Soybeans were harvested in May each year.

2.1.3 Short-term ICLS protocol

The trial ICLS experiment was conducted on an 11 ha site located on Experimental COAMO Farm (Agroindustrial Cooperativa), in Campo Mourão, Paraná State, Brazil (24º05'47" S latitude and 52º21'18" W longitude). It is at an altitude of 630 m, and has a Cfa climate according to the Köeppen classification system. The annual average temperature is 20ºC, and the annual average precipitation is 1570 mm. The soil is an Oxisol, well-drained and dark red. Since 1985, no-tillage crop management was used in the area, and in 2002, the soybean–beef cattle ICLS experiment was established considering two seasons: (1) the winter season (cattle grazing from ~June to August) and (2) the summer season (soybean cropping from ~October to February).

During the winter season, black oat (*Avena strigosa* "common") and Italian ryegrass (*Lolium multiflorum* "common") were planted (60 and 30 kg ha^{-1}, respectively) to form a

mixed pasture. Grazing was forage-based in a continuous stocking system with steers weighing approximately 180 kg (crossbred Angus, Simental, Charolês and Nellore) that entered the pasture system when the forage height reached approximately 35 cm.

Treatments consisted of different sward heights during the winter season, which were determined by monitoring pasture sward heights every 14 days at approximately 20 random locations within each plot and adjusting the stocking rate as necessary, as described previously for the long-term protocol experiment. Targeted pasture vegetation heights were 7, 14, 21 and 28 cm. All treatments were organized in a randomized complete block design with four replications, and plots ranged from 0.5 to 1.7 ha.

At the end of the winter season (middle September), the area was desiccated using glyphosate. In mid-October (summer season), soybean was planted in rows spaced 40 cm apart using a seeding density of 35 seeds m^{-2}. Post-emergent glyphosate was applied in mid-December. Soybeans were harvested in end-February.

2.2. Data collection

2.2.1 Weeds

In the commercial fields, weed communities were surveyed and pasture management heights were measured in 2014 within a 50 m x 40 m area located 100 m away from a field boundary to avoid the field edge effects. Weed data were collected in mid-June 2014 (grazing period). The sampling protocol consisted of recording all species occurring within the 2000 m^{-2} area by walking in a "W" pattern within the plot. The average density of each species was estimated using the Barralis scale of six cover abundance classes described in Fried et al. (2009), where "+" indicates a species found only once in the 2000 m^{-2} area; "1"=less than 1 individual m^{-2}; "2"=1–2 individuals m^{-2}; "3"=3–20 individuals m^{-2}; "4"=21–50 individuals m^{-2}, and "5"=more than 50 individuals m^{-2}. Median values of the abundance class data were

determined following methodology proposed by Trichard et al. (2013). Plants were identified according to Kissmann and Groth (1997) and Lorenzi (2006).

In each experimental unit of the long-term protocol (São Miguel das Missões), weed seedling emergence was quantified for two seasons: at the end of grazing period (November in 2014 and 2015) and during soybean cropping before post-emergent herbicide application (mid-January in 2015 and 2016). The emerged weed seedlings were identified and counted within a 50x50 cm square placed at 14-m intervals in the central area of each experimental unit and distributed along four 56-m transects laid out in the "XX" pattern described by Wiles and Schweizer (2002), to ensure an adequate spatial distribution of measurements. We calculated the population density of each species from the individual number of each species per m^2. Plants were identified according to Kissmann and Groth (1997) and Lorenzi (2006).

2.2.2 Beef production

In the long-term protocol (São Miguel das Missões), animals were weighed at the beginning and end of stocking period, both measured after restricting food and water for 12 h. Average daily gain (kg/animal) was obtained by dividing weight gain by grazing days, while gain per hectare (kg live weight/ha) resulted from multiplying the number of animals per hectare and the average daily gain of animals. Stocking rate, expressed in kg/ha, was calculated by adding the average live weight of the tester animals with the average live weight of each „put and take" animal, multiplied by the number of days they remained in each paddock, and then divided by the number of grazing days. Animal performance was also evaluated during this period. Animal coefficients (i.e. average daily gain, gain per hectare, and stocking rate) in the Short-term ICLS protocol (Campo Mourão) were determined by the same method described for the long-term protocol.

Information about beef production on commercial fields was obtained through a questionnaire sent out to all participating farmers. All evaluated farmers answered 100% of questions regarding a total of 26 fields. All of them had adopted a considerable amount of technology within the farms, which included a digital scale for determining animal weights with 0.1 kg precision. This enabled them to measure the animal weight gain. From their survey responses, we were able to calculate such as average daily gain and gain per hectare.

2.2.3 Soybean yield and glyphosate use

In the long-term protocol (São Miguel das Missões), soybean grain yield and final plant population were evaluated in 2015 and 2016, at the R8 growth stage (full maturity). All plants within a 1.5 linear meter at six random locations per plot. Pods were manually threshed and the seeds were weighed and their moisture content was measured. Grain yield was adjusted to 0.13 kg kg^{-1} moisture content. No difference in grain yield was verified in the evaluated years, so data were averaged over years. Crop yields in the trial ICLS experiment (Campo Mourão) were measured by the same method described for the long-term protocol, with the exception that 2 linear meters was sampled for each sampling unit and there were ten sampling units per plot.

Information about soybean crop yields and farming practices (pesticide and fertilizer use, ploughing and mechanical weed control system) and general information about the farm (number of crops, proportion of land covered and field size) were collected through the questionnaire described earlier. Glyphosate herbicide use was described by the active ingredient concentration and number of applications. For 100% of farms the glyphosate was applied as needed based on a level of weed infestation (individual criteria) and the dose was determined regardless of weed species present.

2.3 Data analysis

2.3.2 Weed diversity and evenness analyses

Shannon's diversity index was estimated for each treatment as follows (Kent and Coker, 1992):

$$H = \sum_{i=1}^{S} \left(\frac{ni}{N}\right)\left(\log_2 \frac{ni}{N}\right)$$

where *N* is the total number of individuals per plot, *ni* refers to the number of individuals per species per plot and *S* describes the total number of species.

The evenness of the species (J) in each treatment was also calculated using Shannon's diversity index as follows (Kent and Coker, 1992):

$$J = \frac{H}{\ln(s)}$$

2.4 Statistical procedures

The relationship between pasture sward height and weed characteristics, glyphosate use, and crop yield was analyzed using linear, quadratic, exponential and segmented mixed models. Farmers, fields, or fields nested within farmers were included as random effects in the models (considering both the short-term and long-term protocols as fields with independent farmers). Farmer inclusion assumed a similar effect across the fields farmed by the same farmer, field inclusion assumed variability across the fields independently of farmers, and finally fields nested within farmers assumed variability between fields for a given farmer. On the segmented models only farmers was used as random effect due to the data availability on the segments. The season (winter and summer) was included as a fixed effect when evaluating weed characteristics. Models were selected based on the Akaïke Information Criterion (AIC), and the likelihood ratio test (LRT) was used to test the significance of fixed and random

effects. Models were fitted by maximizing the restricted log-likelihood. Data were analyzed using R Version 3.1.0 (© 2014 The R Foundation for Statistical Computing).

3. Results

The weed flora assessed in the whole study comprised 23 families and 70 species (appendix A). The impact of sward heights on weed characteristics was analyzed over two seasons (winter and summer), and across fields from Guarapuava, and the long-term protocol of São Miguel das Missões (Fig. 1). As forage allowance increase (i.e., as black oat and Italian ryegrass mixture pasture sward height increased), the weed density decreased exponentially (Fig. 1A). The number of weed species followed similar behavior, but decreased linearly as sward height increased and there was a significant random variability among fields managed by a given farmer (Fig. 1B). There was no sward height effect on weed diversity (Shannon's diversity index) (Fig. 1C). Finally, the evenness index of the weed species decreased as sward height increased along with random variability between farmers (Fig. 1D). There was no effect of season on any weed characteristics evaluated, and neither fields nor farmers affected random variability of weed density and weed diversity index as sward height varied.

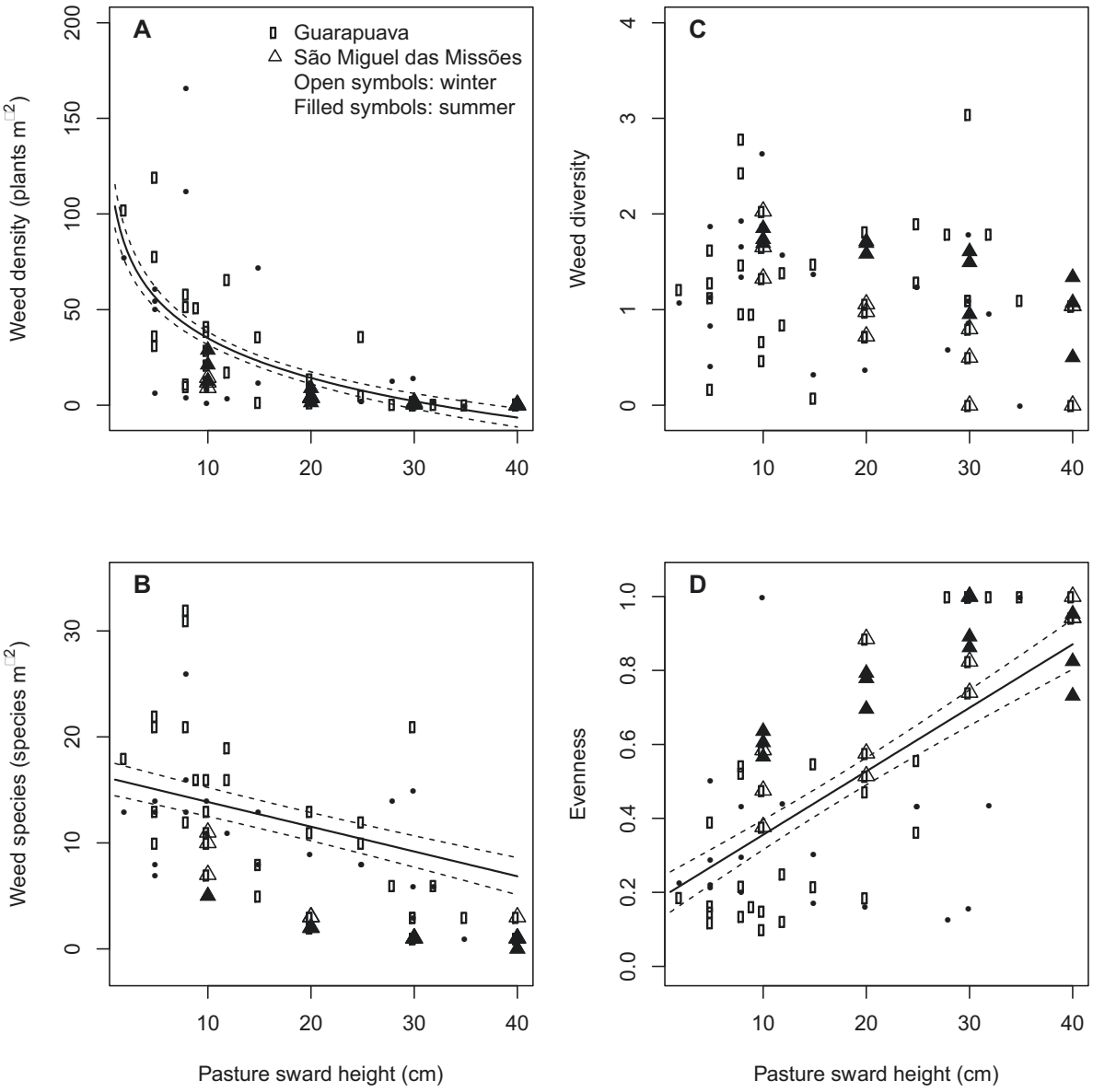

Fig. 1. Changes in winter and summer weed seedling emergence density (A) (y=104.04[±11.3] - 29.96[±4.02] * log(x), p<0.0001, LogLik=-329.1, AIC=664.3, df=72), weed species (B) (y=14.86 [±1.55] - 0.22[±0.04] x, p<0.0001, LogLik=-201.3, AIC=414.6, df=72), Shannon's diversity index (C) (y=1.47 [±0.14] - 0.012[±0.006] x, p>0.05, LogLik=-73.03, AIC=152.1, df=72) and evenness index (D) (y=0.18[±0.06] + 0.017[±0.002] x, p<0.0001, LogLik=-0.51, AIC=9.01, df=72) along a forage allowance (maintained sward height) gradient of mixed black oat and Italian ryegrass winter pasture grazed by cattle in ICLS (pasture sward height [cm]). Dashed lines represent the 95% confidence interval.

The relationship between cover crop residue mass and sward height was measured only on the experimental fields from the Campo Mourão ICLS trial and the long-term protocol of São Miguel das Missões (Fig. 2). The residue mass of grazed or no-grazed (i.e., sole-point of higher achieved residue) cover crop was positively and linearly associated with the cover crop sward height. No random effects of fields or farmers were observed for this relationship.

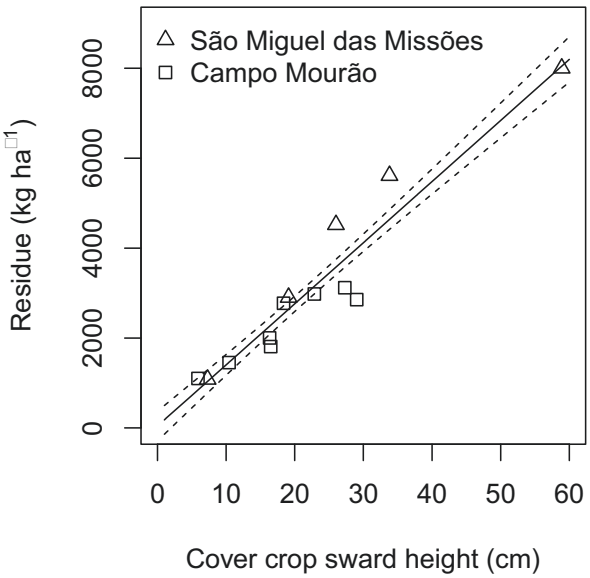

Fig. 2. Changes in residual straw (kg ha^{-1}) (y=45[±330] + 135[±12] x, p<0.0001, LogLik=-91.2, AIC=188.5, df=13) in an ICLS along a forage allowance (maintained sward height) gradient of mixed black oat and Italian ryegrass winter pasture grazed by cattle. Dashed lines represent the 95% confidence interval.

The relationship between glyphosate use and pasture sward height was also evaluated across fields from Guarapuava and the long-term protocol of São Miguel das Missões. The annual glyphosate dose per ha decreased exponentially as sward height increased, with no significant random effects of fields or farmers (Fig. 3).

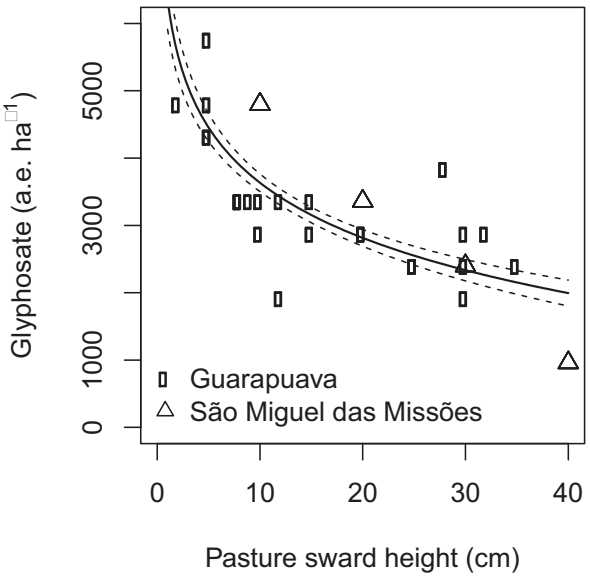

Fig. 3. Changes in applied glyphosate dose (a.e. ha^{-1}) (y=6354 [±441] - 1181.7 [±157] * log(x), p<0.0001, LogLik=-290.5, AIC=587.1, df=38) in an ICLS along a forage allowance (maintained sward height) gradient of mixed black oat and Italian ryegrass winter pasture grazed by cattle. Dashed lines represent the 95% confidence interval.

The glyphosate dose used on the fields was determined as a function of the weed species present. In the winter phase of the long-term protocol (São Miguel das Missões) managed with 10 cm sward heights, the predominant species present were *R. brasiliensis* and *P. tomentosa*, which require approximately 2,400 g a.i. ha^{-1} glyphosate, whereas at sward height of 20 cm, *S. americanum* was predominant, which requires approximately 960 g a.i. ha^{-1} glyphosate, and at higher sward heights (> 20 cm) the rate of glyphosate required for weed control was approximately 480 g a.i. ha^{-1}. In commercial fields we found a similar trend, where lower sward height necessitated higher glyphosate doses, because of the presence of tolerant species such as *Spermacoce latifolia* Aubl., *Cyperus* spp., and *Richardia brasiliensis* Gomes (which required 2880, 2400 and 1920 g i.a. of glyphosate to control, respectively). Our packing analyses (Appendices B) showed that these species occupy niches in pasture sward heights of approximately 8 and 20 cm, respectively, for *S. latifolia* and *R. brasiliensis*.

Considering the generalized occurrence of both species (Appendix A), they necessitated the higher glyphosate doses used in lower swards heights (Fig. 2). *Cyperus* spp., which also require higher glyphosate doses, occurred in only one commercial field with pasture managed at a 5 cm sward height. The glyphosate dose used in pasture swards heights managed at >20 cm was mostly determined by the widespread weeds *Sida rhombifolia* L. in summer and *Stachys arvensis* L. in winter (Appendices A), which require around 1440 and 960 g i.a. of glyphosate, respectively, for effective control.

Beef production and its characteristics were also evaluated as a function of grazing intensity, but these variables were analyzed including fields from the Campo Mourão ICLS trial along with fields from Guarapuava, and the long-term protocol of São Miguel das Missões. Beef production had a quadratic response to sward height, increasing up to a maximum as sward height increased (17.8 cm), but then declined at taller sward height (Fig. 4A). Both the average daily gain (Fig. 4B) and the grazing period (Fig. 4D) showed a similar behavior, increasing up to a maximum as sward height increased (joint points 15.1±2.7 and 6.7±1.3, respectively), but then they slightly declined at taller sward height. The second slopes of the segmented models were not significantly different from 0 ($p>0.1$ for both variables). Both segmented responses to grazing intensity included a significant random variability due to farmers. The stocking rate decreased linearly as sward height increased and there was a random farmer effect (Fig. 4C).

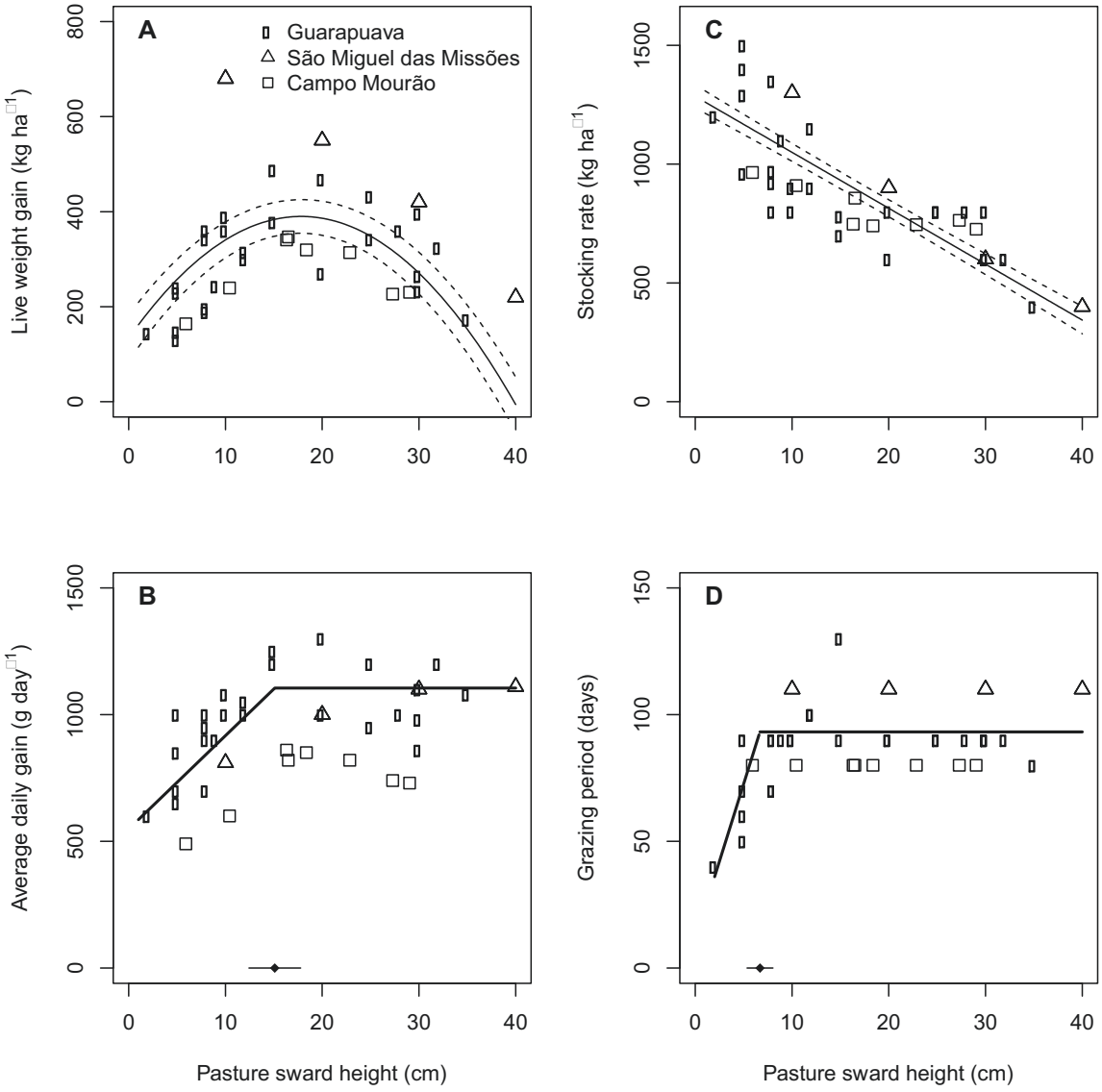

Fig. 4. Changes in cattle live weight gain (A) ($y=134[\pm43] + 28[\pm4.6]\,x - 0.80[\pm0.10]\,x^2$, $p<0.0001$, LogLik=-262.7, AIC=593.3, df=46), average daily gain (B) ($y= 549.1+36.8x$ for $x\leq15.09[\pm2.67]$, $y=1104.62$ for $x>15.09[\pm2.67]$, $p<0.0001$, LogLik=-271.1, AIC=560.7, df=46), stocking rate (C) ($y= 1285[\pm48] - 23.5[\pm1.97]\,x$, $p<0.0001$, LogLik=-287.0, AIC=581.9, df=46), and grazing period (D) ($y= 11.61+12.21x$ for $x\leq6.68$ [±1.33], $y=93.17$ for $x>6.68$ [±1.33], $p<0.0001$, LogLik=-166.8, AIC=348.7, df=46) along a forage allowance (maintained sward height) gradient of mixed black oat and Italian ryegrass winter pasture grazed by cattle in ICLS. Dashed lines represent the 95% confidence interval. Diamonds on x axes represent break points, and its associated horizontal bars denote standard errors.

Summer soybean yield was affected by winter sward height across all the evaluated fields (Fig. 5). Soybean yield increased with increasing sward height up until 10.8±1.8, but decreased at taller sward heights (second slope was not significantly different from 0, p>0.01), and the response had a random variability due to farmers.

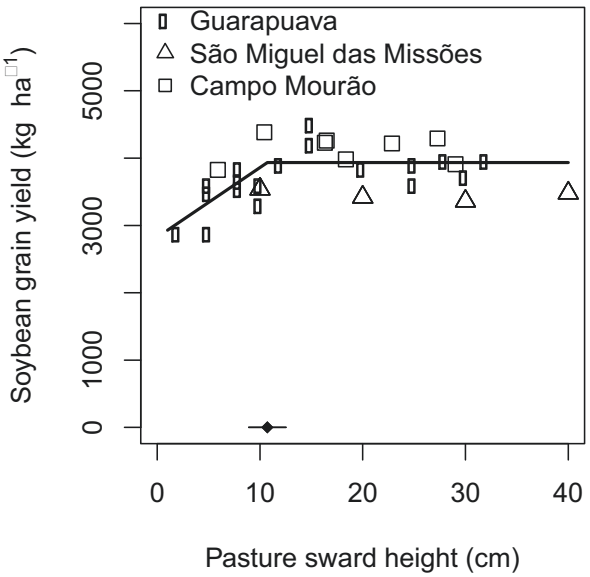

Fig. 5. Summer soybean yield response (y= 2824+103.7x for x≤10.7 [±1.82], y=3935 for x>10.7 [±1.82], p<0.0001, LogLik=-243.0, AIC=501.7, df=38) to a forage allowance (maintained sward height) gradient of mixed black oat and Italian ryegrass winter pasture grazed by cattle in ICLS.

4. Discussion

Analyzing data from commercial fields comes at the cost of various uncontrolled factors (e.g., system management, environmental factors, and weed pools) that may increase dispersion of responses; however, to strengthen accuracy of analysis we used two protocols (shorter and longer-term experiments) that provided robust data under controlled conditions. Treatment effects were reasonably similar between commercial and experimental fields, but for some of the variables, there was significant variability associated with different fields,

farmers, or fields for a given farmer. Regression models were developed under the assumptions described earlier, and resultant predictions and confidence intervals are shown in Figures 1-5.

4.1 Weed density and diversity in an ICLS

A recent report emphasized the role of conserving weed species diversity in arable fields because of its role in supporting ecosystem services (Gaba et al., 2016). Weed presence in croplands stimulates a long-running debate among ecologists, chemical companies, agricultural advisors, and farmers. All of them play a pivotal role in society, but the conflicts regarding herbicide usage invoke issues ranging from maintaining high crop yield, risk to the environmental quality and food security, to biodiversity maintenance. To help resolve this conflict, alternative weed control methods can play a pivotal role in reducing weed density below economic thresholds while maintaining weed diversity, thus conserving important ecosystem services such as feeding pollinators.

Our study showed that varying sward heights (representing different forage allowances) didn"t affect weed diversity, although sward heights drastically shifted weed community composition and structure. Smaller sward height reduced the number of dominant weeds (Fig. 1D), however, lower grazing intensity increased species richness (Fig. 1B) and maintained weed diversity (Fig. 1C). Schuster et al. (2016) showed that increasing grazing intensity in ICLS also increased the seed bank population density and richness. Despite effective and useful weed control by herbicides in an ICLS (Schuster et al., 2015), our results here showed that using smaller sward height dramatically reduced weed density (Fig. 1A), and maintained weed diversity in ICLS (Fig. 1C). Organic and agroecological systems designed to minimize synthetic pesticide inputs should consider taller sward height as a strategy to both reduce potential crop-weed competition and maintain weed diversity.

The amount of residual straw increased linearly as the pasture sward height increased (Fig. 2). Teasdale and Mohler (1993) showed the importance of straw in reducing weed emergence, which may explain why weed occurrence decreased exponentially as the sward height increased (Fig. 1A). In order to achieve a 50% reduction in emergence of herbicide-resistant weeds promoted by residue added to the soil surface in no-tillage systems, Webster et al. (2016) found a required residue amount of 5.2 ton ha^{-1} for *Amaranthus palmeri*, and Schuster et al. (2016) reported a required residue amount of 3.2 ton ha^{-1} for *Conyza canadensis*. Based on our results that the residue amount is strongly and positively related to the cover crop sward height, maintaining ICLS swards heights of 38 and 23 cm would be enough to promote 50% suppression in emergence of *Amaranthus palmeri* and *Conyza canadensis*, respectively. The packing analyses for *C. canadensis* (Appendix B) in our study confirmed results for its suppression in taller swards since the niche for this species was from 0 to 25.8 cm of pasture sward height (Appendix A).

4.2 Glyphosate use in an ICLS

Crop management practices (e.g., crop rotation, tillage practices) can change weed communities in arable lands (Cardina et al., 2002; De Cauwer et al., 2010); however, there are a surprising lack of reports that have analyzed the weed community changes in response to herbicides (like glyphosate) applied on an as-needed basis for control of specific weeds. The demand for glyphosate use in croplands is associated with species present in the overall weed population; for example, as described by the Roundup® commercial registration in Brazil, the recommended dose (g a.e.) per ha ranges from 240 (e.g., for controlling *Brachiaria plantaginea*) to 5760 (e.g., for controlling *Guadua angustifolia*). Our results showed that amount of glyphosate input required decreased exponentially as sward height increased (Fig.

3), wherein the glyphosate dose used in each field was determined as a function of the weed species present.

Farmers have been relying heavily on glyphosate for weed control, but they are experiencing a rapid increase in the number of weed species resistant to glyphosate in their fields (Heap, 2017). One reason for this is repeated application and high glyphosate doses used by farmers, which accelerates the resistance process. This use pattern has not only resulted in a reduction of the weed control spectrum of glyphosate, but is exacerbated by a greater potential risk of glyphosate residues in food (Beckie, 2011). Therefore, farmers need to use lower doses of glyphosate and use alternative herbicide modes of action to prolong the utility of glyphosate as a highly effective herbicide. Our study demonstrated that using high forage allowances (>17.8 cm of sward height), it was possible to reduce glyphosate demand when compared with lower forage allowances (<10.7 cm of sward height) in ICLS (Fig. 3). The decisions regarding a glyphosate management strategy in ICLS should consider increase forage allowances as a way to increase glyphosate use efficiency and sustainability in following years.

4.3 Beef production in an ICLS

Our results indicate that maintaining sward heights at 17.8 cm is optimal to maximize meat production in an ICLS (i.e. 389.9 kg ha^{-1} of live weight gain; Fig. 4A), and this response is similar to those reported by Cassol (2003), Aguinaga et al. (2006), Lopes et al. (2008) and Wesp (2010). The optimal sward height that achieved greatest beef production was strongly influenced by the average live weight gain (animal performance; Fig. 4B), for which the minimum optimal sward height was 15.1±2.7 cm (non-significant second slope), despite increasing stocking rate at lower sward heights (Fig. 4C). It is well known among grazing ecology researchers that animal performance in forage-based grazing is associated with

herbage intake (Poppi et al., 1981; Hodgson, 1982). Moreover, Amaral et al. (2012) and Carvalho et al. (2013) showed that excessively small or excessively tall sward heights could limit daily herbage intake due to difficulties related with herbage bite apprehension. Specifically, smaller sward heights may have had higher proportions of stems + sheaths, which constrain the bite mass (Fonseca et al., 2012). In contrast, as described in Mezzalira et al. (2014), taller sward heights might reduce bite mass and it is considered a complex destabilizing mechanism that can generate detrimental spatial heterogeneity in both sward height and mass, which was not verified in our results.

The grazing period was strongly reduced at very lower forage allowance (sward heights lower than 6.7±1.3 cm; Fig. 4D). Pasture managed with small swards heights did not provide the same plant regrowth capacity after grazing as higher swards heights, possibly because of its smaller leaf-area index. Consequently, low sward heights did not enable a similar grazing period as greater sward heights because of plant exhaustion. Briefly, when plant photosynthesis becomes severely limited due to unavailable leaf area, plants draw on stored energy reserves in tissues from roots and other parts. When the stored energy is depleted, plant growth ceases and rapid regrowth potential is minimized.

Currently, world annual meat consumption per capita is 41.9 kg including bovine, poultry, pig and ovine meat (FAO, 2001), and as the world population is projected to reach 10 billion in 2050 (FAO, 2016), several reports suggested that a diet change from meat-based to cereal-based food is required (Cassidy et al., 2013). However, changing diets is not an easy task in many countries around the world because of traditional cultures. According to our results, winter grazing of a cover crop maintained at an optimum sward height of 17.8 cm has the potential to provide meat production from 1 hectare that would sustain the average annual meat demand of 3.7 persons according to the FAO average consumption mentioned above (protein source based exclusively on beef) and based on a 40% dressing percentage of the

animals [FAO, 1972]. Considering that only 45% of global meat production is derived from bovine (FAO 2001), then one hectare of the ICLS could provide the beef component of the per capita meat consumption of 8.2 persons.

In Southern Brazil, 19.3 million hectares are being cultivated with summer crops (mostly soybean) under no-tillage management. However, the area cultivated with grain crops during winter season usually reaches less than 25% of the summer-cultivated area, with the remaining area seeded mostly with non-grazed cover crops (CONAB, 2016). This implies that there is a large area (~75% of 19.3 million hectares) that could be intensified with ICLS containing grazed cover crops (mostly black oat and ryegrass due to the favorable environmental conditions). Using the best sward height management observed from our results (i.e. 17.8 cm) and assuming an annual meat demand of 41.9 kg per capita based on the FAO estimate mentioned previously, it would be theoretically possible to provide enough beef to satisfy the annual meat demand of 53.8 million people based exclusively on beef, or 119.2 million people if beef provided 45% of the meat in a diversified protein diet.

4.4 Soybean yield in an ICLS

Grazed cover crops provide a direct source of revenue for farms (McKenzie et al., 2016), however farmers still have concerns about grazing consequences for subsequent crop yields. The positive and negative implications of grazing cover crops in ICLS are well-established. Moraes et al. (2014) reviewed 23 papers that evaluated grain yields from crops in succession with winter-grazed cover crops (mostly black oat and Italian ryegrass mixture), and most of these studies showed greater summer grain yields in grazed than non-grazed areas. However, a few studies that managed winter pastures under lower forage allowance (i.e. <12 cm sward height of black oat and Italian ryegrass mixture) did not have the same positive responses on summer crop grain yield, possibly because of grazing-associated

problems such as soil compaction and nutrient depletion. Our results agree that black oat and Italian ryegrass pastures managed with sward heights less than 12 cm resulted in severe negative consequences for both soybean yields and beef production.

Our results showed the optimal sward height in winter pasture that resulted in the greatest soybean yield the following summer was higher than 10.7±1.8 cm (Fig. 5). Beyond the factors already stated above about reduction in grain yields promoted by sward heights lower than 12 cm, higher sward heights correspond to a smaller cattle load, and as a consequence, greater residue amounts left on the soil surface at the end of the grazing season. Greater residues increase soil coverage and promote other advantages often related to no-tillage systems (e.g. greater water retention and reduced weed emergence). Moreover, moderate forage allowance (i.e. >17.8 cm sward height according to our results) can result in improved soil quality for subsequent crops in ICLS (Sulc and Franzluebbers, 2014) and improved nutrient cycling in the entire ICLS (Carvalho et al., 2010). This emphasizes that the magnitude of these benefits is often determined by the grazing intensity, and it is intrinsically related to the flux of organic mass and energy among the soil-plant-animal-atmosphere components.

Cassidy et al. (2013) proposed to re-examine agricultural productivity as the number of people fed per hectare rather than tons of yield per hectare, and for this the author used available food calories from crops for people to be nourished (which is 985,500 or 2700 calories per person per year or day, respectively). If beef and soybean were to be used exclusively for direct human consumption and based on energy values of 2445 cal/kg for beef and 3596.5 cal/kg for soybean (Cassidy et al. 2013), our results showed it is theoretically possible to feed 14.2 people per hectare per year. Furthermore, 21.1% more people could be fed using the optimal grazing sward height of 17.8 cm compared to the lowest sward height of 2 cm.

5. Conclusion

Using a moderate forage allowance (i.e., maintaining a 17.8 cm sward height) in a winter mixed pasture of black oat and Italian ryegrass in ICLS allowed significant reductions in glyphosate use and significant increases in soybean yield and beef production when compared with lower forage allowance (i.e. <10.7 cm pasture sward height). Overall results suggest that closely managing the forage allowance (and thus sward height) during the grazing phase of ICLS is a key component for meeting the goal of reducing herbicide inputs while maximizing beef and soybean production.

6. Acknowledgements

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7. References

Aguinaga, A.A.Q., Carvalho, P.C.F., Anghinoni, I., Dos Santos, D.T., Freitas, F.K., Lopes, M.T., 2006. Production of beef steers grazing oat plus annual ryegrass pasture managed at different heights. R. Bras. Zootec. 35, 1765–1773.

Amaral, M.F., Mezzalira, J.C., Bremm, C., Trindade, J. K., Gibb, M.J., Suñe, R.W.M., Carvalho, P.C.F., 2012. Sward structure management for a maximum short-term intake rate in anual ryegrass. Grass and Forage Science. 68, 271–277.

Barralis, G. 1976. Méthode d'étude des groupements adventi- ces des cultures annuelles. In: Ve. Colloque International sur l'Ecologie et la Biologie des Mauvaises Herbes. Dijon, FR. pp. 59-68

Barzman, M.S., Dachbrodt-Saaydeh, S., 2011. Comparative analysis of pesticide action plans in five European countries. Pest Manag Sci. 67, 1481–1485.

Beckie, H.J., 2011. Herbicide-resistant weed management: focus on glyphosate. Pest Manag. Sci. 67, 1037–1048.

Benbrook, C.M., 2016. Trends in glyphosate herbicide in the United States and globally. Environmental Sciences Europe. 28, 0-15.

Boissinot, F., Mézière, D., Bretagnolle, V., Munier-Jolain, N.M., 2011. Réduire l"usage des herbicides en grandes cultures. Phytoma. 649, 39-44.

Cardina, J., Herms, C.P., Doohan, D.J., 2002. Crop rotation and tillage system effects on weed seedbanks. Weed Sci. 50, 448-460.

Carvalho, P.C.F., 2013. Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? Tropical Grasslands – Forrajes Tropicales. 1, 137-155.

Carvalho, P.C.F.; Anghinoni, I.; Moraes, A.; Souza, E.D.; Sulc, R.M.; Lang, C.L.; Flores, J.P.C.; Lopes, M.L.T.; Silva, J.L.; Conte, O.; Wesp, C.L.; Levien, R.; Fontaneli, R.S.; Bayer, C., 2010. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. Nutrient Cycling in Agroecosystems, 88, 259–273.

Cassidy, E.S.; West, P.C.; Gerber, J.S.; Foley, J.A., 2013 Redefining agricultural yields: from tonnes to people nourished per hectare. Environmental Research Letters. 2013;8:034015. doi: 10.1088/1748-9326/8/3/034015.

Cassol, L.C., 2003. Relação solo-planta-animal num sistema de integração lavoura pecuária em semeadura direta com calcário na superfície. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (PhD thesis). p. 140.

CONAB - National Supply Company (Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira-grãos. http://www.conab.gov.br.

Cauwer, B., Van den Berge, K., Cougnon, M., Bulcke, R., Reheul, D., 2010. Weed seedbank responses to 12 years of applications of composts, animals urines or mineral fertilizers. Weed Res. 50, 425–435.

FAO 1972 Technical Conversion Factors for Agricultural Commodities (Rome: Food and Agriculture Organisation) (www.fao.org/economic/the-statistics-division-ess/ methodology/methodology-systems/technical-conversionfactors-for-agricultural-commodities/ar/).

FAO 2011 Food Outlook: Global Market Analysis (Rome: Food and Agriculture Organisation) (www.fao.org/docrep/014/al978e/al978e00.pdf).

FAO 2016 How to Feed the World in 2050 (Rome: Food and Agriculture Organisation) (www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf).

Fried, G., Chauvel, B., Reboud, X., 2009. A functional analysis of large-scale temporal shifts from 1970 to 2000 in weed assemblages of sunflower crops in France. Journal of Vegetation Science. 20, 49-58.

Fonseca, L., Carvalho, P.C.F, Mezzalira, J.C., Bremm, C. Galli, J.R., Gregorini, P., 2013. Effect of sward surface height and level of herbage depletion on bite features of cattle grazing *Sorghum bicolor* swards. J. Anim. Sci., 91, 4357–4365.

Gaba, S., Reboud, X., Fried, G., 2016. Agroecology and conservation of weed diversity in agricultural lands, Botany Letters. 163, 351-354.

Heap, I., 2017. The International Survey of Herbicide Resistant Weeds. Online. Accessed 1 April 2017. Available at www.weedscience.org

Herrero, M., Thornton, P.K., Notenbaert, A.M., Wood, S., Msangi, S., Freeman, H.A., Bossio, D., Dixon, J., Peters, M., Van de Steeg, J., Lynam, J., Rao, P.P., Macmillan, S., Gerard, B., McDermott, J., Seré, C., Rosegrant, M., 2010. Smart investments in sustainable food production: revisiting mixed crop-livestock systems. Science. 327, 822-824.

Hodgson, J., 1982. Influence of sward characteristics on diet selection and herbage intake by the grazing animal. In: Hacker, J.B. (Ed.), Nutritional Limits to Animal Production from Pastures: Proceedings of an International Symposium. St. Lucia, Queensland, pp. 153–166.

Hunt, J.R., Swan, A. D., Fettell, N. A., Breust, P. D., Menz, I. D., Peoples, M. B., Kirkegaard, J. A., 2016. Sheep grazing on crop residues do not reduce crop yields in no-till controlled traffic farming systems in an equi-seasonal rainfall environment. Field Crops Research. 196, 22–32.

IARC (International Agency for Research on Cancer). IARC Monographs Volume 112: evaluation of five organophosphate insecticides and herbicides. 2015. https://www.iarc.fr/en/media centre/iarcnews/pdf/MonographVolume112.pdf

Keulen, H., Schuman, H., 2004. Crop-Livestock system: old wine in new bottles? In: Fisher, T. et al.(Eds), New Directions for a Diverse Planet. Proceedings of the IV international Crop Science Congress. Australia.

Kissmann, K.G., Groth, D., 1997, Plantasinfestantes e nocivas.BASF, São Paulo. Obra em 3 volumes.

Kunrath, T.R., Cadenazzi, M., Brambilla, D.M., Anghinoni, I., Moraes, A., Barro, R.S., Carvalho, P.C.F., 2014. Management targets for continuously stocked mixed oat X

annual ryegrass pasture in a no-till integrated crop-livestock system. Eur. J. Agron. 57, 71-76.

Lechenet, M., Deytieux, V., Antichi, D., Aubertot, J.N., Bàrberi, P., Bertrand, M., Cellier, V., Charles, R., Colnenne-David, C., Dachbrodt-Saaydeh, S., Debaeke, P., Doré, T., Farcy, P., Fernandez-Quintanilla, C., Grandeau, G., Hawesj, C., Jouyk, L., Justes, E., Kierzek, R., Kudsk, P., Lamichhane, J.R., Lescourret, F., Mazzoncini, M., Melander, B., Messéan, A., Moonen, A.C., Newton, A.C., Nolot, J.M., Panozzo, S., Retaureau, P., Sattin, M., Schwarz, J., Toqué, C., Vasileiadis, V.P., Munier-Jolaina, N., 2016. Diversity of methodologies to experiment Integrated Pest Management in arable cropping systems: Analysis and reflections based on a European network. European Journal of Agronomy. 83, 86-99.

Lemaire, G., Franzluebbers, A., Carvalho, P.C.F., Dedieu, B., 2013. Integrated crop–livestock systems: strategies to achieve synergy between agricultural production and environmental quality. Agr. Ecosyst. Environ. 190, 4-8.

Lopes, M.L.T., Carvalho, P.C.F., Anghinoni, I., Santos, D.T., Kuss, F., Freitas, F.K., Flores, J.P.C., 2008. Crop–livestock integration system: performance and carcass quality of superprecoce beef steers finished in oat and ryegrass pasture managed under different heights. Cienc. Rural. 38, 178–184.

Lorenzi, H.M., 2006. de Identificação e Controle de Plantas daninhas, sixth ed. Nova and Publishing House, Odessa, São Paulo: Instituto Plantarum.

McKenzie, S.V., Goosey, H.B., O'Neill, K.M., Menalled, F.D. 2016. Integration od sheep grazing for cover crop termination into market gardens: Agronomic consequences of an ecologically based management strategy. Renewable Agriculture and Food Systems. 1-14.

Mezzalira, J.C., Carvalho, P.C.F., Fonseca, L., Bremm, C., Cangiano, C., Gonda, H.L., Laca, E.A., 2014. Behavioral mechanisms of intake rate by heifers grazing swards of contrasting structures. Applied Animal Behaviour Science, 153, 1-9.

Moraes, A., Carvalho, P.C.F., Lustosa, S.B.C., Lang, C.R., Deiss, L., 2014. Research on Integrated Crop-Livestock Systems in Brazil. Revista Ciência Agronômica. 45, 1024-1031.

Mott, G.E., Lucas, H.L., 1952. The design, conduct and interpretation of grazing trials on cultivated and improved pastures. 6° Intl Grassld. Cong, p. 1380.

Nie, Z.; McLean, T.; Clough, A.; Tocker, J.; Christy, B.; Harris, R.; Riffkin, P.; Clark, S.; McCaskill, M., 2016. Benefits, challenges and opportunities of integrated crop-livestock systems and their potential application in the high rainfall zone of southern Australia: A review. Agriculture, Ecosystem and Environment.235, 17-31.

Poppi, D.P., Minson, D.J., Ternouth, J.H., 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. 1. The voluntary intake, digestibility and retention time in the reticulo-rumen. Aust. J. Agric. Res. 32, 99–108.

Schuster, M.Z., Pelissari, A., Szymczak, L.S., Lustosa, S.B.C., Moraes, A., 2015. Chemical control of white clover in Soybean Crops. Planta Daninha. 33, 1-5.

Schuster M.Z., Pelissari, A., Moraes, A., Harrison, S.K., Sulc, R.M., Lustosa, S.B.C., Anghinoni, I., Carvalho, P.F.C., 2016. Grazing intensities affect weed seedling and the seed bank in an integrated crop-livestock system. Agriculture, Ecosystem and Environment. 232, 232-239.

Soil Survey Staff, 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys, in: USDA Natural Resource Conservation Service Agriculture Handbook No. 436. U.S. Government Printing Office, Washington, DC.

Sulc, R.M.; Franzluebbers, A.J., 2014. Exploring integrated crop-livestock systems in different ecoregions of the United States. European Journal of Agronomy. 57,21-30.

Teasdale, J.R., Mohler, C.L., 1993. Light transmittance, soil-temperature, and soil-moisture under residue of hairy vetch and rye. Agronomy Journal. 85, 673–680.

Trichard, A., Alignier, A., Chauvel, B., Petit, S., 2013. Identification of weed community traits response to conservation agriculture. Agriculture, Ecosystems and Environment. 179, 179-186.

Webster, T.M., Simmons, D.B., Culpepper, A.S., Grey, T.L., Bridges, D.C., Scully, B.T. 2016. Factors affecting potential for Palmer amaranth (*Amaranthus palmeri*) suppression by winter rye in Georgia, USA. Field Crops Res. 192, 103-109.

Wesp, C.L., 2010. Sistema de integração lavoura-pecuária: desempenho de novilhos superprecoces e variabilidade espacial do pasto. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Wiles, L., Schweizer, E., 2002. Spatial dependence of weed seed banks and strategies for sampling. Weed Sci. 50, 595-606.

Appendix A

Table 1. Weed species relative density (emerged seedling m^{-2}) and frequency (%) found in farm fields in Southern Brazil.

Species	Family	Winter		Summer	
		D	F	D	F
Alternanthera philoxeroides (Mart.) Griseb.	Amaranthaceae	0.004	3.84		
Amaranthus viridis L.	Amaranthaceae	0.004	3.84	0.004	18.18
Ambrosia tenuifolia L.	Asteraceae	0.04	7.69	0.02	27.27
Axonopus barbigerus (Kunth.) Hitchc.	Poaceae	0.004	3.84		
Baccharis uncinella DC.	Asteraceae	0.004	3.85		
Bidens pilosa L.	Asteraceae	0.019	19.23	0.09	31.81
Boerhavia diffusa L.	Nyctaginaceae	0.008	7.69		
Bowlesia incana Ruiz & Pav.	Apiaceae	0.011	11.53		
Brachiaria brizantha (Hochst.) Stapf.	Poaceae	0.007	7.69	0.527	22.72
Brachiaria plantaginea L.	Poaceae	0.05	15.38	9.90	90.90
Bromus catharticus Vahl	Poaceae	0.011	11.53		
Carex spp.	Cyperaceae	0.004	3.84		
Chamaesyce hyssopifolia (L.) Small	Euphobiaceae	0.007	7.69	0.004	18.18
Chenopodium album L.	Chenopodiaceae	0.003	3.84	0.009	18.18
Ciclospermum leptophyllum (Pers.) Sprague	Apiaceae	0.007	3.84	0.004	18.18
Commelina benghalensis L.	Commelinaceae	0.003	3.84	0.09	40.90
Conyza bonariensis (L.) cronquist	Asteraceae	0.034	3.84		
Conyza canadensis (L.) Cronquist.	Asteraceae	3.496	69.23	1.8	63.64
Coronopus didymus (L.) Sm.	Brassicaceae	0.015	15.38	0.004	18.18
Cuphea carthagenensis (Jacq.) J.F. Macbr	Lythraceae	0.004	3.84	0.004	18.18
Cyperus esculentus L.	Cyperaceae	0.003	3.84	0.018	3.84
Cyperus rotundus L.	Cyperaceae	0.007	3.84		
Dichondra repens L.	Convolvulaceae	0.012	11.54	0.009	22.72
Digitaria horizontalis Willd.	Poaceae			2.32	68.18
Echinochloa crus-pavoni (Kunth) Schultes	Poaceae			0.004	18.18
Eleusine indica (L.) Gaertn	Poaceae	0.023	23.07	0.904	68.18
Euphorbia heterophylla L.	Euphorbiaceae	0.019	19.23	2.25	68.18
Facelis apiculata L.	Asteraceae	0.669	30.76	0.072	22.72
Facelis retrusa (Lam.) Schultz-Bip.	Asteraceae	0.065	11.53		
Gnaphalium spicatum Lam.	Asteraceae	0.807	84.61	0.077	27.27
Hypochoeris brasiliensis (Less.) Griseb.	Asteraceae	0.457	19.23	0.006	18.18
Ipomoea sp.	Convolvulaceae	0.003	3.84	0.577	59.09

* Blank fields represent where species were not found in winter or summer.

….Continuation of table 1

Species	Family	Winter		Summer	
		D	F	D	F
Leonurus sibiricus L.	Lamiaceae	0.088	19.23	0.004	18.18
Malvastrum coromandelianum (L.) Garcke	Malvaceae	0.038	3.84		
Murdannia nudiflora (L.) Brenan	Commelinaceae	0.015	15.38		
Oxalis corniculata L.	Oxalidaceae	1.93	11.53	0.009	22.72
Paspalum paniculatum L.	Poaceae	0.003	3.84		
Pennisetum clandestinum L.	Poaceae	0.004	3.84		
Plantago major L.	Plantaginaceae	0.007	7.69		
Plantago tomentosa Lam.	Plantaginaceae			0.004	18.18
Polygonum convolvulus L.	Polygonaceae	0.004	3.84	0.004	18.18
Polygonum punctatum Elliott.	Polygonaceae			0.004	18.18
Portulaca oleracea L.	Portulacáceas			0.004	18.18
Raphanus raphanistrum L.	Brassicaceae	4.78	80.76	4.02	77.27
Raphanus sativus L.	Brassicaceae	0.04	3.84		
Richardia brasiliensis Gomes	Rubiaceae	0.503	30.76	0.709	63.64
Rumex obtusifolius L.	Polygonaceae	0.011	11.53		
Senecio brasiliensis L.	Asteraceae	0.234	57.69	0.69	50.00
Setaria parviflora (Poir.) Kerguélen	Poaceae	0.004	3.84		
Sida cordifolia L.	Malvaceae	0.003	3.84		
Sida rhombifolia L.	Malvaceae	0.292	38.46	6.27	81.81
Silene gallica L.	Caryophyllaceae	0.004	3.84		
Solanum americanum L.	Solanaceae	0.123	30.76	0.018	31.81
Solanum pseudocapsicum L.	Solanaceae	0.004	3.84	0.013	27.27
Solanum sisymbriifolium L.	Solanaceae	0.081	42.31	0.03	36.36
Solanum viarum L.	Solanaceae	0.004	3.84		
Soliva pterosperma (Juss.) Less.	Asteraceae	0.073	19.23	0.004	18.11
Sonchus oleraceus L.	Asteraceae	0.154	50.00	0.018	31.81
Spergula arvensis L.	Caryophyllaceae			0.004	18.18
Spermacoce latifolia Aubl.	Rubiaceae	0.065	11.53	0.041	54.55
Stachys arvensis L.	Lamiaceae	7.454	76.92	0.222	31.81
Stellaria media (L.) Vill	Caryophyllaceae	4.807	38.46		
Taraxacum officinale (L.) Weber	Asteraceae	0.007	7.69	0.005	18.18
Vernonia polysphaera L.	Asteraceae	0.007	7.69	0.004	18.11
Veronica peregrina (Kunth) Pennell	Scrophulariaceae	2.57	76.92		
Xanthium strumarium L.	Asteraceae	0.081	11.53	0.004	18.18

* Blank fields represent where species were not found in winter or summer.

Table 2. Weed species relative density (emerged seedling m^{-2}) and frequency (%) found in long-term ICLS protocol.

Species	Family	Winter		Summer	
		D	F	D	F
Brachiaria plantaginea (Link) Hitchc.	Poaceae	0.07	50	4.52	100
Conyza bonariensis (L.) Cronquist	Asteraceae	0.05	25		
Conyza canadensis (L.) Cronquist	Asteraceae	3.57	100	0.58	75
Cyperus esculentus L.	Cyperaceae			0.03	50
Digitaria horizontalis Willd.	Poaceae	0.09	25	0.32	75
Echium plantagineum L.	Boraginaceae	0.08	50		
Eleusine indica L. Gaertn	Poaceae	0.05	25	0.15	50
Facelis retrusa (Lam.) Schultz-Bip.	Asteraceae	0.71	25		
Gnaphalium spicatum Lam.	Asteraceae	0.07	50		
Heteranthera reniformis Ruiz & Pav.	Pontederiaceae	0.24	75	0.39	100
Ipomea purpurea (L.) Roth	Convolvulaceae			0.05	25
Nothoscordum gracile (Aiton.) Steam.	Alliaceae	0.05	50		
Phyllanthus tenellus Roxb.	Euphorbiaceae	0.30	50	0.10	75
Plantago tomentosa Lam.	Plantaginaceae	2.38	25	0.24	25
Richardia brasiliensis Gomes	Rubiaceae	0.86	25	1.30	75
Senecio brasiliensis Less.	Asteraceae	1,38	75	0.09	75
Sida rombifolia L.	Malvaceae			0.26	100
Solanum americanum Mill.	Solanaceae	0.03	50	0.06	50
Soliva pterosperma (Juss.) Less.	Asteraceae	3.57	25		
Stachys arvensis L.	Lamiaceae	0.09	50		
Taraxacum officinale F.H. Wigg.	Asteraceae	0.67	25		
Xantium strumarium L.	Asteraceae			0.03	50

* Blank fields represent where species were not found in winter or summer.

Species packing analyses

A mathematical model of species packing in an n-dimensional environmental space represents niches as hyperspheres, based on a single environmental parameter as a point in n-dimensional space (Gauch et al., 1974). To estimate the effective range of grazed cover crop sward height that created niches for *Spermacoce latifolia* Aubl., *Richardia brasiliensis* Gomes, *Sida rhombifolia* L. and *Stachys arvensis* L. we fitted models to species abundances along a sward height gradient. The algorithm is the same as for the Gaussian function in the nonlinear regression model:

$$y = ae^{\frac{(x-b)^2}{2c^2}}$$

The curve has mean *b* and standard deviation *c*.

Initial estimates of *y* and *b* maximum values followed Gauch et al. (1974) to solve the equation for *c*. Initial estimation of optimum and tolerance based on the weighted average, followed by a nonlinear optimization by the Levenberg-Marquardt method was used to solve least squares curve-fitting problems (Transtrum and Sethna, 2012):

$$[J^T W J + \lambda\, diag(J^T W J)]h_\mu = J^T W(y - \hat{y})$$

The algorithm was described by Lourakis (2005). The sward height range niche for each species was determined by abscissa intersection from the ordination number of maximum and minimum tolerance in the fitted model. The data for this analysis was from commercial fields (Guarapuava). We excluded from this analysis the data from long-term protocol because the same grazing intensities (sward heights) maintained for many years had direct effects on weed seed bank density and composition (Schuster et al., 2016), and this factor can under- or over-estimate species niches along the sward height gradient.

Results of species packing analyses:

Stachys arvensis L. and *Sida rhombifolia* L. weren‟t having niche along sward height gradient of grazed cover crop (the model no adjusted, and these weeds have distributed similarity along sward height gradient of grazed cover crop in an ICLS).

Table 3 - Estimated optima and niches (cm) for those species in sward height gradient of grazed cover crop in an ICLS and the dose of glyphosate for control each species.

Species	Sward height of occurrence (cm)		Dose of glyphosate
	Optimal emerged seedlings	Niche range	
Conyza canadensis (L.) Cronquist.	00.0	00.0 - 25.8	GR**
Spermacoce latifolia Aubl.	06.7	04.8 - 08.2	2880*
Richardia brasiliensis Gomes	16.6	12.0 - 22.0	1920*

** Glyphosate-resistant
*Grams per hectare of isopropylamine salt of glyphosate required to control species according to Original Roundup® label.

References

Lourakis A. I. M., 2005. A Brief Description of the Levenberg-Marquardt Algorithm Implemened by Levmar. Foundation for Reseach and Technology, Crete-Greece.

Schuster et al., 2016. Grazing intensities affect weed seedling and the seed bank in an integrated crop-livestock system. Agriculture, Ecosystem and Environment. 232, 232-239.

Gauch, H. G.; Chase, G. B.; Whittaker, R. H., 1974. Ordination of vegetation samples by Gaussian species distributions. Ecology. 55, 1382-1390.

Transtrum, M. K. and Sethna, J. P., 2012. Improvements to the Levenberg-Marquardt algorithm for nonlinear least-squares minimization," Preprint submitted to Journal of Computational Physics. 30.

CHAPTER 4

Previous biomass production drives weed abundance in grassland-cropping rotation [1]

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Previous biomass production drives weed abundance in grassland-cropping rotation

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Abstract

The knowledge about practices that reduce weed abundance in arable lands is suitable to integrated weed management planning, and previous studies showed that including grassland into the cropping system for a period longer than three years dramatically reduces weed abundance. Here, we modelled the weed abundance in a grassland-cropping rotation that results from previous biomass production and demonstrated that if only the length of time with temporary grassland introduced into cropping systems is taken into account, it is not enough to reduce weed abundance. This study was carried out for 12 years, and the treatments consisted of sequences of maize, wheat and barley with different precedent grassland periods (three or six years with different amounts of nitrogen fertilization) and two control treatments with continuous grassland or cereal-based rotation. Our results showed that harvested biomass production a year before better explained the weed abundance than the treatments. The competitive exclusion of weeds by crop or grassland biomass production that stabilizes the amount of weed abundance in arable lands is dependent on if, in each previous year, the crop or grassland achieves the equilibrium level of 24.3 and 4.7 tons ha^{-1} of biomass production, respectively. Therefore, if the previous year reaches a biomass production level that is higher than the equilibrium level, the crop or grassland contributes to the reduction of weed abundance. However, in years where the biomass production is below the equilibrium level, there is an increase in weed abundance in the next year. Furthermore, the equilibrium level of biomass production changes as a function of weed traits, such as life history and leaf type as well as morphology.

Keywords: weed ecology, weed management, plant population dynamics, integrated crop-livestock systems, sod-based rotation.

Introduction

Worldwide, weeds pose a risk to successful grassland and crop production. Highly effective and sustainable weed control has a pivotal role in meeting the global food security. In this context, excessive use of herbicides has resulted in serious environmental and ecological issues.

Grassland-cropping rotation has been found to reconcile agricultural production and environmental quality (Lemaire et al., 2015). Previous studies showed that these rotations had several impacts in the weed community trajectory and resulted in far fewer problematic weeds known to cause yield loss (Meiss et al., 2010) as well as a reduction in the costs and risks of herbicide use (Lehnhooff et al., 2017; Tracy and Davis, 2009). However, another study on grassland-cropping systems reported a 30-fold increase in weed pressure and a yield reduction of 51.2% compared to a sole-cropping system (Miller et al., 2015). Therefore, factors that drive weed dynamics in grassland-cropping systems are not well understood and thus this information will be suitable in helping to redesign integrated weed management in these systems.

Here, we investigated the weed abundance dynamics on grassland-cropping rotations over 12 years. Specifically, we investigated whether weed abundance in both crops and grasslands changes with the duration and fertilization of the temporary grassland included in the rotation. We fit alternative models to these data to test if the effect of the fertilization and duration could be mediated by variations of grassland and crop biomass production. We propose a model expressing the abundance of weeds as a function of the culture in place and the biomass produced in previous years by the grassland and crop. Finally, we identify how this relationship is affected by weed traits.

Materials and Methods

Site description

The cropping systems with the temporary grasslands used for the long-term experiment of this study were located on SOERE ACBB (Observatory and Experimental System for Environmental Research - Agroecosystems, Biogeochemical Cycles, and Biodiversity; http://www.soere-acbb.com/) at the INRA Lusignan in Poitou-Charentes, France (46º25‟13” N latitude; 0º07‟29” E longitude, 151 m altitude). This site has an oceanic climate with a summer drought, with an average temperature of 12ºC and a yearly average precipitation of 750 mm. The soil is a rubefied brown earth on clay, with traces of ferruginous shell.

Starting in 2005, the trial experiment was established with treatments designed as sequences of maize, wheat and barley with different grassland rotational periods. The grasslands were seeded mid-September with a mixture of three grass species: rye grass (*Lolium perenne* cv. Milca: 5 kg ha^{-1}), fescue (*Festuca arundinacea* cv. Soni: 10 kg ha^{-1}) and orchard grass (*Dactylis glomerata* cv. Ludac: 12 kg ha^{-1}). Post-emergent herbicide was applied in the time of grassland installation. For each plot, grasslands were mowed to 5–7 cm stubble height and harvested (Haldrup) around three times each year (varied from 1 to 5 depending on biomass production) and herbage was removed from the field. The first cut occurred in the spring (April). They were fertilized after each mowing.

The treatments were designed in four blocks with individual plots of 4000 m^2 each: (1) a cereal-based rotation, which included the cultivation as a sequence of maize/wheat/barley; (2) a grassland-cropping rotation, which had 3 years of grassland prior to the start of the experiment and then the treatment began with 3 years of cultivated maize/wheat/barley followed by three years of grasslands and finally ended with the cultivation of

maize/wheat/barley; (3 and 4) a grassland-cropping rotation, which, prior to the start of the experiment, had six years of grassland with either high nitrogen fertilization or reduced nitrogen fertilization (respectively for 3 and 4) and then began with six years of grassland in place followed by maize/wheat/barley; and (5) continuous grassland.

Data collection

Field sampling of weed abundance

In each experimental unit in the field, weed abundance was determined during the maize/wheat/barley cropping before post-emergent herbicide application and in the grassland before the first cut. The abundance of weeds was estimated using the adapted Barralis scale for a circumference of 0.25 m^{-2}, with class "0" being attributed to no weeds in 0.25 m^{-2}, class "1" for 1 individual weed in 0.25 m^{-2}, "2" for 2-5, "3" for 6-12, and "4" for more than 12. In each plot, the sampling was conducted within 13 points placed at 12-m intervals in the central area (i.e., excluding 5 m of edge) and distributed along two 72-m transects laid out in an "X" pattern.

Field sampling of crop and grassland biomass

The production of each plot was expressed in dry matter mass per unit area (MS t ha^{-1}). The biomass harvested on each of the plots was estimated at three different sample points whenever mowing or crop harvest occurred and the resulting samples were used to measure the dry matter content. The samples were taken at the Haldrup (experimental harvester equipped with an on-board weighing system). The surface area of each sample was at least 7.50 m^2, which corresponds to a Haldrup passage of 1.50 m of cutting bar and a length of 5 m.

The length of the sample had to be greater in situations where biomass production was lower or heterogeneous. The height of the Haldrup's cutting bar was adjusted to the height of the mower, approximately 6-7 cm above the ground.

Data management

Initial data gathering was performed in spreadsheets, and the assembling and consolidation of the data were performed in R, notably the R packages data.table (Dowle et al., 2015) and test that (Wickham, 2011) for merging and consistency checks.

General modelling procedures

All analyses were performed in the R software for statistical computing version 3.1.3 (R Development CoreTeam, 2015). Unless otherwise specified, we describe the abundance of the weeds in a field as the sum of the abundances at the 13 sampled points by a negative binomial distribution with a logarithmic link to account for the discrete nature of the counts and some over-dispersion in the data. To estimate the effect of the treatments and components of the management, we used either fix effect models as implemented in the MASS R package (Venables, 2002) or mixed linear models as implemented in the lme4 package (Bates et al., 2015). We compared the models using the Akaike‟s Information Criterion (AIC) as provided by the regression packages (Sakamoto and Akaike, 1978). To prevent inaccurate statistical estimates due to a temporal autocorrelation of the observations in successive years, we checked that no autocorrelation of the residuals over time could be detected (with acf in the R package itsadug). Significance of the differences between factor levels, such as the different treatments, according to the aforementioned models, was carried out with pairwise

comparisons using the Holm–Bonferroni adjustment method as implemented in the R package lsmeans (Lenth, 2015).

The relevance of the models to describe the variability in the data was tested with the "goodness of fit" test by Fisher (Fisher, 1924). We used several indicators to characterize the quality of the predictions from fitted models: the Root Mean Square Error of prediction (RMSEp) and the bias as implemented in the hydroGOF package (Zambrano-Bigiarini, 2014) as well as the R^2 and the Spearman rank correlation as implemented in the Hmisc package (Frank and Harrell, 2016).

Model of weed control by culture biomass

Hereafter, we propose a model of connecting the weed flora abundance to the harvested biomass of the previous year. We assume that for each year, if the harvested biomass, B, is higher than a culture-specific equilibrium level, S_c, then the weed flora, W, the following year tends to decrease. In contrast, the weed flora increases if the cultivated biomass is less than S_c. For this reason, at year T, the estimate of the raised weed flora ($\widehat{W}_T$) is proportional to the ratio of the equilibrium level and the harvested biomass in the previous year, modulated by a power coefficient that decreases with time:

$$\widehat{W}_T \propto \left(\frac{S_{c(T-1)}}{B(T-1)}\right)^{a} \times \left(\frac{S_{c(T-2)}}{B(T-2)}\right)^{\frac{a}{2}} \times \ldots \times \left(\frac{S_{c(0)}}{B(0)}\right)^{\frac{a}{T}} = \prod_{t=1}^{T} \left(\frac{S_{c(T-t)}}{B(T-t)}\right)^{\frac{a}{t}}$$

This can be expressed logarithmically to obtain a linear formulation:

$$\log\left(\widehat{W}_T\right) = I + \sum_{t=1}^{T} \frac{a}{t} \log\left(\frac{S_{c(T-1)}}{B(T-t)}\right)$$

In the above equation, the biomass over time and the inverse of time are separable:

$$\log(\widehat{W_T}) = I + a\sum_{t=1}^{T} -\frac{\log(B(T-t))}{t} + \sum_{c}\left(a.\log(S_c)\sum_{t=1}^{T}\frac{1_c(t)}{t}\right)$$

where $1_c(t)$ is the indicator function of the presence of culture c on year t. The terms of this linear expression are identifiable with the terms of a negative binomial regression with a logarithmic link:

$$W_T \sim NB(\iota + \alpha k + \Gamma N)$$

where ι is the intercept of the regression corresponding to I in the initial linear expression. Here, we account for the group of fields, P, and the current crop in the plot, C, either with fixed or random effects:

$$\iota(P,C) = \iota_P + \iota_C$$

The factor k accounts for previously harvested biomasses: $k = -\sum_{t=1}^{T}\frac{\log(B(T-t))}{t}$, and $\alpha = a$ is the corresponding coefficient in the regression. N is a vector of crop factors corresponding to the sum of the inverse of the elapsed time since the crop was present: $N_c = \sum_{t=1}^{T}\frac{1_c(t)}{t}$ and Γ is the vector of corresponding regression coefficients per culture: $\Gamma_c = a.log(S_c)$.

Independent of the categorical factors lumped into the intercept ι, once k and N are calculated, the equilibrium level of harvested biomass for each culture c is simply given by the exponential of the ratio of the regression coefficients:

$$S_c = \exp\left(\frac{\Gamma_c}{\alpha}\right)$$

As the rotation is always the same for cash crops, in the following we only distinguish grasslands from cash crops, grouping maize, wheat and barley together.

As the distribution of the residuals against the predicted value was not homogeneous, we also tested a square root link. It significantly improved the fit but had little impact on the estimated equilibrium levels.

Results

Effects of grassland duration and management on weed abundance

The weed abundance in the grassland-cropping rotations substantially changed and had seemingly important divergent trajectories over the years depending on their management (Figure 1A). To assess the statistical significance of the differences in weed abundance between treatments, we modelled the weed abundance as a function of the treatment and the culture in place, controlling for the field block. Over the 12 years of the study, the weeds were less abundant in treatments that included six years with temporary grasslands in the rotation if and only if the grassland was well fertilized with nitrogen (Figure 1B). In contrast, six years of temporary grassland with reduced nitrogen fertilization in the rotation resulted in higher weed abundances, similar to those observed in rotations without grasslands (Figure 1B). Rotations with well fertilized grasslands over a greater number of years had lower weed abundances (treatment 2 vs. 3). Note that the effect of the treatments remained significant despite accounting for the culture in place (i.e., grassland, maize, wheat or barley), which also had a strong effect on weed abundance: weed abundance in maize and wheat was approximately 3 times greater than in grassland and barley (Figure 1C).

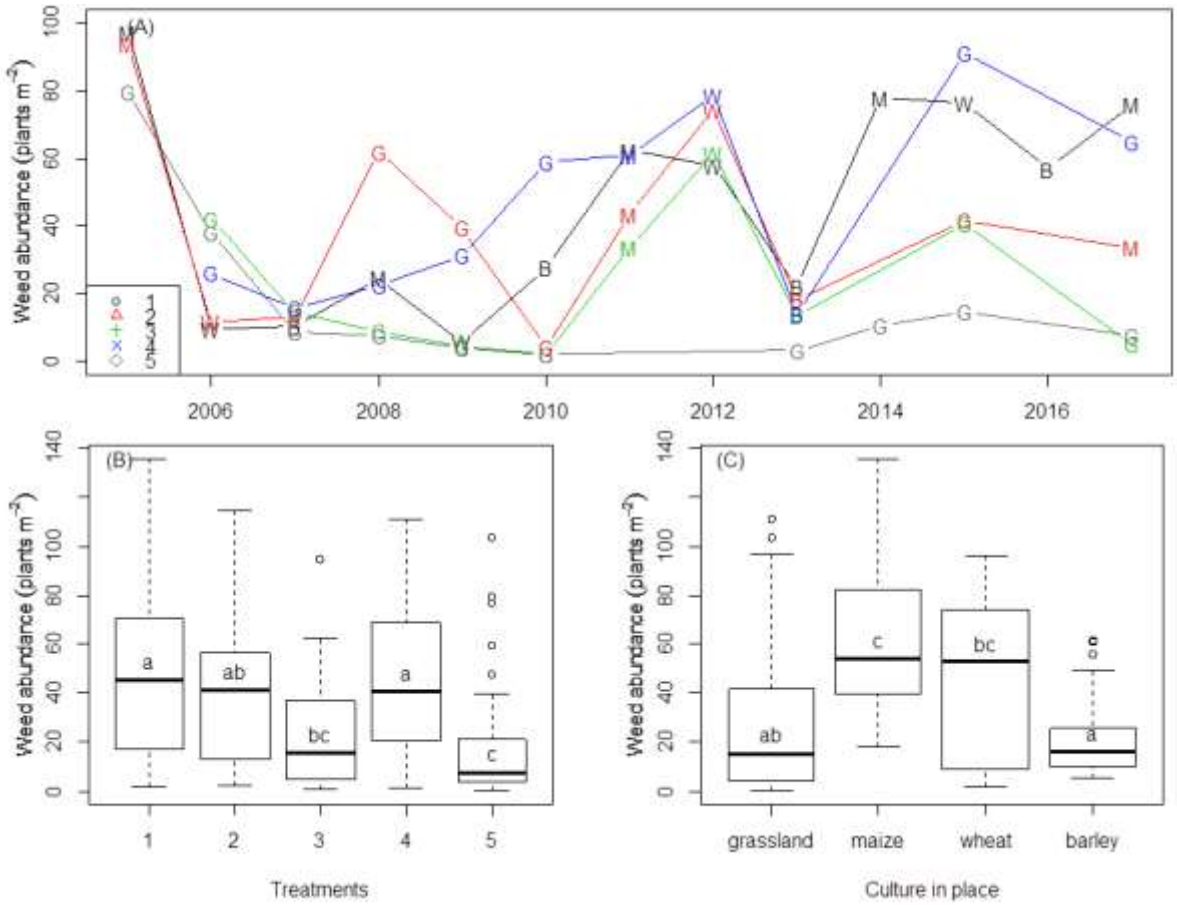

Figure 1. Weed abundance dynamics (A) and effects of treatments (B) and culture in place (C) on weed abundance (plants m^{-2}) during the 12 years of the grassland-cropping rotation experiment. Uppercase letters in panel (A) indicate: G=grassland, M=maize, W=Wheat and B=Barley. Different lowercase letters (a, b, c) in panels (B) and (C) indicate significant differences between the system treatments and culture according to the pairwise comparisons with the Holm–Bonferroni adjustment method ($P< 0.05$).

To further illustrate the weed reduction effect of well fertilized grasslands both in the grasslands and for the following crops, we analysed specific triennia in which the cultures were the same in the different treatments with the same model. In 2011-2013, all treatments had crops in place (except treatment 5, the permanent grassland), and these crops came after grasslands (except for treatment 1, the system with only crops). The crop fields with six years of well fertilized grassland in previous years had a statistically significant reduction in weed

abundance of 25 to 50% compared to fields with only crops in the previous years (Figure 1A and Table 1). The system with grassland for only three years and the system that had six years of grassland with reduced fertilization did not have significantly fewer weeds than the system with only crops (Table 1). In 2014-2015, all treatments had grassland in place (except for the system with only crops), and these grasslands came after crops. The grassland reintroduction in fields that previously had grassland managed with reduced nitrogen fertilization had higher weed abundance than fields that had previous grasslands that were well fertilized with nitrogen, independent of where the temporary grassland occurred in the rotations (Table 1).

Table 1. Effects of treatments on weed abundance (GLM coefficient "Estimate") in the triennium with crop after grassland (2011-2013) or grassland after crop (2014-2016) cultivation.

Treatments	Crop after grassland (2011-2013)	Grassland after crop (2014-2016)
1*	0 a	0 a
2	-0.141 a	-0.682 b
3	-0.396 b	-0.724 b
4	-0.064 a	0.124 a
5	-1.789 c	-1.866 c

*Reference treatment. Different lowercase letters (a, b, c) indicate significant differences between the system treatments according to the pairwise comparisons with the Holm–Bonferroni adjustment method ($P < 0.05$).

Model of weed control by culture biomass evaluation and consistency

For a comprehensive understanding of the effects of the different treatments, we decomposed those in a biomass production of grassland and crops, and the predicted weed abundance from the proposed model that connected the weed flora abundance to the harvested biomass of the previous years (Figure 2) correctly matched the observed values with

acceptable R^2 (higher than 0.57) and with correct ranking (Spearman correlation coefficients higher than 0.75). The mean error of prediction (RMSE) was approximately 19 weed plants m^{-2} and the bias test demonstrated that there was a tendency to an overestimation of 4.2%.

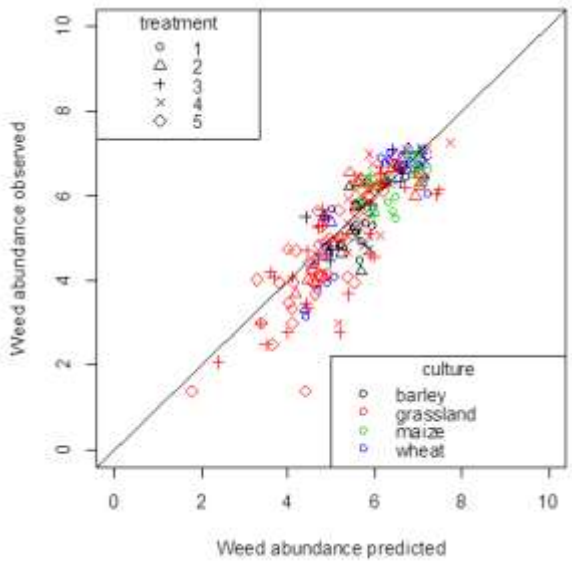

Figure 2. Model results showing the abundance of weeds predicted vs. the abundance of weeds observed in the grassland-cropping rotation (link= sqrt).

Weed traits response to previous grassland biomass production

The model that predicted weed abundance in the grassland-cropping rotation revealed that the equilibrium levels of biomass that stabilized weed abundance were 24.3 and 4.7 ton ha^{-1} for crop and grassland, respectively, and that weed traits heavily altered these equilibrium levels of biomass (Table 2). Weeds with a perennial life history, monocotyledon leaf type and rosette, grass or climbing morphology demanded more biomass production of grassland to reduce the weed abundance the following year. Conversely, the upright weed morphology proved to be a weak competitor with grassland and was reduced with a low production of

grassland biomass. However, weeds that were annuals and had an upright or creeping morphology were strong competitors with crops (Table 2).

Table 2. Equilibrium levels of biomass production required to change (reduce) the weed abundance the following year as a function of species traits.

Type	Traits	Ton ha^{-1}	
		Crop	Grassland
Life history	Perennial	11.4	5.5
	Annual	44.2	4.4
Leaf type	Monocotyledons	56.0	6.4
	Dicotyledons	16.1	3.1
Morphology	Rosette	7.9	4.4
	Upright	112.5	0.1
	Grass	39.1	6.7
	Climbing	14.9	6.3
	Creeping	83.1	2.1
General	-	24.3	4.7

Discussion

This field trial (i.e., SOERE-ACBB) was designed to increase our understanding of the long-term impacts of temporary grasslands in arable cropping systems (Lemaire et al., 2005). Our study provides new insight about weeds in the grassland-cropping rotation in several ways. Previous studies broadly reported that weed infestation is reduced with the inclusion of grassland in arable lands (e.g., Schoofs and Entz, 2000; Bellinder et al., 2004; Teasdale et al., 2004; Albrecht, 2005; Heggenstaller and Liebman, 2006; Norris and Ayres, 1991; Entz et al., 1995; Andersson and Milberg, 1996; Gill and Holmes, 1997; Clay and Aguilar, 1998; Ominski et al., 1999; Sjursen, 2001; Cardina et al., 2002; Sosnaski, 2006; Cavigelli, 2008; Hiltbrunner et al., 2008; Tracy and Davis, 2009; Meiss et al., 2010). Here, we showed that the biomass production of the previous culture determined the future weed abundance in the entire system. Our study is the first, to our knowledge, to determine that an equilibrium level

exists with a specific biomass production of crop or grassland and that a reduction in weed abundance in arable lands is dependent on achieving a biomass production above this equilibrium level the year before. Furthermore, we demonstrated that weed biological traits, such as life history and leaf type as well as the morphology, are involved in determining if the weed community is more or less persistent to competitive exclusion by culture biomass.

Weed abundance reduction by the introduction of grassland in arable lands

The model was built with a 12-year dataset, which confers an important robustness to the equilibrium level coefficients of biomass production derived from the data. The impacts of biomass on weeds are probably produced by several factors affecting different phases of the weed life cycle, including seed germination and emergence, plant survival and vegetative growth, and seed production and seed survival.

Generally, our grassland-cropping system showed that crop biomass competition was not effective for weed control, with demands of crop biomass production many times higher than capacity. However, the results derived from our data are limited to conditions of seed rate, row spacing, crop cultivar and row direction, and previous studies have demonstrated that it is necessary to utilize these strategies to improve the competitive ability of crops and achieve an effective weed control (Sardana et al., 2017).

Despite the potential to improve the competitive ability of crops, the grassland biomass competition was found to be more effective than crops in managing weeds. A high quantity of grassland biomass production and the absence of soil tillage favour the establishment of weed-suppressive mulch (Wiens et al., 2006) and grasslands that re-close the canopy more quickly after mowing can offer higher resistance to invader or weed growth and fecundity (Milbau et al., 2003). High vegetation cover also changes the light quality,

temperature and humidity on the soil surface, all of which impact weed germination (Huarte and Arnold, 2003). Moreover, the impact of competition is probably highest for young weed plants (Magda et al., 2006).

Seed predation during the grassland phase may also play a role in reducing future weed abundance. The absence of soil tillage allows more weed seeds to stay exposed on the soil surface, and weed seed predation rates in perennial forage are positively related to higher biomass cover (Meiss et al., 2010). The permanent vegetation cover may constitute a more stable habitat compared to annual crops that might favour the presence of different seed predators (Van Klinken, 2005). Weed seed survival on the soil surface might not only be reduced by seed predation but also by seed decay (Gardarin et al., 2010).

Weed trait response to grassland biomass production in arable lands

Perennial weed species, compared with annuals, required higher grassland biomass production to reduce their abundance. Perennial species might be better adapted to competition with grasslands; several previous studies reported that grasslands favoured perennial over annual weed species (Ominski et al.,1999; Hiltbrunner et al., 2008). For the leaf type, monocotyledons showed higher survival rates and quicker regrowth after grassland cutting compared to dicotyledonous species (Meiss et al., 2008), which is in agreement with our results.

When considering the morphology of weeds, the rosette and grass types were more competitive with grasslands than other morphologies. Conversely, for the upright morphology, the inclusion of grassland into the cropping system is all that is needed to reduce weed abundance due to the lower threshold of grassland biomass production required by this morphology (i.e., 0.1 ton ha^{-1}; Table 2). Our results support the findings from previous studies

that demonstrated that perennial forage suppressed the upright and climbing species when compared to the creeping and rosette species (Teadasle et al., 2004; Heggenstaller and Liebman, 2006; Lian et al., 2006).

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Declaration of interest

The authors have no conflicts of interest to declare.

References

Albrecht H. (2005) Development of arable weed seedbanks during the 6 years after the change from conventional to organic farming. Weed Res. 45 (5), 339-350. DOI: 10.1111/j.1365-3180.2005.00472.x.

Andersson T.N., Milberg P. (1996) Weed performance in crop rotations with and without leys and at different nitrogen levels, Ann. Appl. Biol. 128, 505–518.

Bellinder R.R., H.R. Dillard, D.A. Shah. (2004) Weed seedbank community responses to crop rotation schemes. Crop Prot. 23 (2), 95-101. DOI: 10.1016/S0261-2194(03)00174-1.

Cardina J., Herms C.P., Doohan D.J. (2002) Crop rotation and tillage system effects on weed seedbanks, Weed Sci. 50, 448–460.

Cavigelli M.A., Teasdale J.R. , Conklin A.E. (2008) Long-term agronomic performance of organic and conventional field crops in the mid-Atlantic region, Agron. J. 100, 785–794.

Clay S.A., Aguilar I. (1998) Weed seedbanks and corn growth following continuous corn or alfalfa, Agron. J. 90, 813–818.

Entz M.H., Bullied W.J., KatepaMupondwa F. (1995) Rotational benefits of forage crops in Canadian prairie cropping systems, J. Prod. Agric. 8, 521–529.

Gardarin, A., Dürr, C., Mannino, M. R., Busset, H. &Colbach, N. (2010b). Seed mortality in the soil is related to the seed coat thickness. Seed Science Research20: 243-256.

Gill G.S., Holmes J.E. (1997) Efficacy of cultural control methods for combating herbicide-resistant Lolium rigidum, Pestic. Sci. 51, 352–358.

Heggenstaller A.H., M. Liebman. (2006) Demography of Abutilon theoprasti and Setaria faberi in three crop rotation systems. Weed Res. 46 (2), 138-151.

Hiltbrunner J., Scherrer C., Streit B., Jeanneret P., Zihlmann U., Tschachtli R. (2008) Long-term weed community dynamics in Swiss organic and integrated farming systems, Weed Res. 48, 360–369.

Huarte H.R., R.L.B. Arnold. (2003) Understanding mechanisms of reduced annual weed emergence in alfalfa. Weed Sci. 51 (6), 876-885.

Lehnhoff, E., Miller, Z., Miller, P., Johnson, S., Scott, T., Hatfield, P., Menalled, D., 2017. Organic Agriculture and the quest for the holy grail in water-limited ecosystems: managing weeds and reducing tillage intensity. Agri. 7, 4–8.

Lemaire G, Gastal F, Franzluebbers A, Chabbi A. Grassland–Cropping Rotations: An Avenue for Agricultural Diversification to Reconcile High Production with Environmental Quality. Environmental Management (2015) 56:1065–1077.

Lemaire, G., Wilkins, R., Hodgson, J., 2005. Challenges for grassland science: managing research priorities. Agriculture, Ecosystems & Environment 108, 99–108. doi:10.1016/j.agee.2005.01.003

Lian J.Y., W.H. Ye, H.L. Cao, Z.M. Lai, S.P. Liu. (2006) Effects of periodic cutting on the structure of the Mikania micrantha community. Botanical Studies 47 (2), 185-190.

Magda D., M. Duru, J. Huguenin, B. Gleizes. (2006) Impact of shading and cutting on the demography and composition of Mimosa pudica L., a ligneous weed species of tropical grasslands. Grass Forage Sci. 61 (1), 89-96.

Meiss, H., Médiène, S., Waldhardt, R., Caneill, J., Bretagnolle, V., Reboud, X., Munier-Jolain, N., 2010. Perennial lucerne affects weed community trajectories in grain crop rotations. Weed Res., 50, 331–340.

Meiss, H., Munier-Jolain, N., Henriot, F., Caneill, J., 2008. Effects of biomass, age and functional traits on regrowth of arable weeds after cutting. J. Plant Dis. Prot. 21, 493–499.

Meiss, H.; Bonnot, R.; Strbik, F.; Waldhardt, R.; Caneill, J.; Munier-Jolain, N. Cutting and competition reduce weed growth: additive or interactive effects? XIIIème dijon – 8 - 10 septembre 2009 colloque international sur la biologie des mauvaises herbes

Meiss, H.; Le Lagadec, L.; Munier-Jolain, N.; Waldhardt, R.; Petit, S. Weed seed predation increases with vegetation cover in perennial forage crops. Agriculture, Ecosystems and Environment 138 (2010) 10–16

Milbau, A.; Nijs, I.; Van Peer, L.; Reheul, D.;De Cauwer, B. 2003. Disentangling invasiveness and invasibility during invasion in synthesized grassland communities. New Phytologist, 159: 657–667.

Miller, Z., Menalled, F.D., Sainju, U.M., Lenssen, A.W., 2015. Integrating sheep grazing into cereal-based crop rotations: spring wheat yields and weed communities. Agronomy Journal. 107, 104-112.

Norris R.F., Ayres D. (1991) Cutting Interval and Irrigation Timing in Alfalfa: Yellow Foxtail Invasion and Economic Analysis, Agron. J. 83, 552–558.

Ominski P.D., Entz M.H., Kenkel N. (1999) Weed suppression by Medicago sativa in subsequent cereal crops: a comparative survey, Weed Sci. 47, 282–290.

Sardana, V.; Mahajan, G.; Jabran, K.; Chauhan, B.S (2017). Role of competition in managing weeds: An introduction to the special issue. Crop Protection 95, 1-7.

Schoofs A., M.H. Entz. (2000) Influence of annual forages on weed dynamics in a cropping system. Can. J. Plant Sci. 80 (1), 187-198.

Schuster, M.Z., Pelissari, A., de Moraes, A., Harrison, S.K., Sulc, R.M., Lustosa, S.B.C., Anghinoni, I., Carvalho, P.C.F., 2016. Grazing intensities affect weed seedling emergence and the seed bank in an integrated crop–livestock system. Agric. Ecosyst. Environ. 232, 232–239.

Sjursen H. (2001) Change of the weed seed bank during the first complete six-course crop rotation after conversion from conventional to organic farming, Biol. Agric. Hortic. 19, 71–90.

Sosnoskie L.M., Herms N.P., Cardina J. (2006) Weed seedbank community composition in a 35-yr-old tillage and rotation experiment, Weed Sci. 54, 263–273.

Teasdale J.R., R.W. Mangum, J. Radhakrishnan, M.A. Cavigelli. (2004) Weed seedbank dynamics in three organic farming crop rotations. Agron. J. 96 (5), 1429-1435.

Tracy, B.F., Davis, A.S., 2009. Weed biomass and species composition as affected by an integrated crop–livestock system. Crop Sci. 49, 1523–1530.

Van Klinken R.D. (2005) Total annual seed loss on a perennial legume through predation by insects: The importance of within-season seed and seed feeder dynamics. Austral. Ecol. 30 (4), 414-425.

Wiens M.J., M.H. Entz, R.C. Martin, A.M. Hammermeister. (2006) Agronomic benefits of alfalfa mulch applied to organically managed spring wheat. Can. J. Plant Sci. 86 (1), 121-131.

Lenth, R.V. (2016) Least-squares means: The R Package lsmeans. Journal of Statistical Sofware, 69, 1-33.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48.

R Development CoreTeam, 2015. R: A language and environment for statistical computing.

Wallach, D., 2006. Evaluating crop models, in: Wallach, D., Makowski, D., Jones, J.W. (Ed.), Working with Dynamic Crop Models. Elsevier, Amsterdam,11–53.

Van de Wiel, M.A., Di Bucchianico, A., 2001. Fast computation of the exact null distribution of Spearman‟s rho and Page's L statistic for samples with and without ties. J. Stat. Plann, 92, 133–145.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

SAKAMOTO, Y; AKAIKE, H. "Analysis of cross classified data by AIC". Annals of the Institute of Statistical Mathematics. 1978, vol 30, num. 1, p. 185--197.

Fisher, R. A. (1924) “The conditions under which the x2 measures the discrepancy between observation and hypothesis,” Journal of the Royal Statistical Society, 87, 442-450.

M Dowle, A Srinivasan, T Short, S Lianoglou with contributions from R Saporta and E Antonyan (2015). data.table: Extension of Data.frame. R package version 1.9.6. http://CRAN.R-project.org/package=data.table.

Hadley Wickham. testthat: Get Started with Testing. The R Journal, vol. 3, no. 1, pp. 5--10, 2011.

Russell Lenth (2015). lsmeans: Least-Squares Means. R package version 2.20-23. http://CRAN.R-project.org/package=lsmeans.

Frank E; Harrell Jr, with contributions from Charles Dupont and many others. (2016). Hmisc: Harrell Miscellaneous. R package version 3.17-4. http://CRAN.R-project.org/package=Hmisc.

Mauricio Zambrano-Bigiarini (2014). hydroGOF: Goodness-of-fit functions for comparison of simulated and observed hydrological time series. R package version 0.3-8. http://CRAN.R-project.org/package=hydroGOF.

FINAL CONSIDERATIONS

Weed management in ICLS has relied heavily on herbicides to avoid crop yield losses caused by crop-weed competition. Long-term reliance on herbicides as the primary and often sole management method for weed control is problematic due in part to the high potential for such selection pressures to result in herbicide-tolerant or herbicide-resistant weed populations. A multitactical and flexible approach that provides a buffered system of weed control and prevents or delays development of problematic weed populations is a desirable goal for any weed management system. The knowledge gained from this thesis on processes related to ICLS-weed interactions underlines the importance of grazing management on ICLS to manage weed populations, notably with the use of lower grazing intensities (i.e., high forage allowances) and higher biomass production practices. However, managing weed populations requires the use of several agronomic practices beyond grazing management, both during the grassland and in subsequent crops. In addition, the grazing management allows other ecosystem services provided by ICLS to benefit, such as reducing the impact of phytosanitary pressure on the environment and having a positive effect on biodiversity. This thesis focused on weed populations; therefore, future studies should perform a multicriteria analysis on ICLS of weed harmfulness for crop production (crop yield loss, technical harvest problems, harvest pollution, field infestation, and crop disease increase) and weed-related biodiversity (weed species richness and equitability, trophic resources for birds, insects and pollinators).

REFERENCES

Barzman, M.S., Dachbrodt-Saaydeh, S., 2011. Comparative analysis of pesticide action plans in five European countries. Pest Manag Sci 67:1481–1485.

Busi, R., Vila-Aiub, M.M., Beckie, H.J., Gaines, T.A., Goggin, D.E., Kaundun, S.S., Lacoste, M., Neve, P., Nissen, S.J., Norsworthy, J.K., Renton, M., Shaner, D.L., Tranel, P.J., Wright, T., Yu, Q., Powles, S.B., 2013. Herbicide-resistant weeds: from research and knowledge to future needs. Evolut. Appl. 6, 1218–1221.

Fedoroff, N.V., Battisti, D.S., Beachy, R.N., Cooper, P.J., Fischhoff, D.A., Hodges, C.N., Knauf, V.C., Lobell, D., Mazur, B.J., Molden, D., Reynolds, M.P., Ronald, P.C., Rosegrant, M.W., Sanchez, P.A., Vonshak, A., Zhu, J.K., 2010. Radically rethinking agriculture for the 21st century. Science 327, 833–834.

Food and Agriculture Organisation [FAO], 2016. How to Feed the World in 2050. Food and Agriculture Organisation, Roma.

Herrero, M., Thornton, P.K., Notenbaert, A.M., Wood, S., Msangi, S., Freeman, H.A., Bossio, D., Dixon, J., Peters, M., van de Steeg, J., Lynam, J., Rao, P.P., Macmillan, S., Gerard, B., McDermott, J., Sere, C., Rosegrant, M., 2010. Smart investments in sustainable food production: revisiting mixed crop-livestock systems. Science 327, 822–825.

Lechenet, M., Deytieux, V., Antichi, D., Aubertot, J.N., Bàrberi, P., Bertrand, M., Cellier, V., Charles, R., Colnenne-David, C., Dachbrodt-Saaydeh, S., Debaeke, P., Doré, T., Farcy, P., Fernandez-Quintanilla, C., Grandeau, G., Hawes, C., Jouy, L., Justes, E., Kierzek, R., Kudsk, P., Lamichhane, J.R., Lescourret, F., Mazzoncini, M., Melander, B., Messéan, A., Moonen, A.C., Newton, A.C., Nolot, J.M., Panozzo, S., Retaureau, P., Sattin, M., Schwarz, J., Toqué, C., Vasileiadis, V.P., Munier-Jolain, N., 2017. Diversity of methodologies to experiment integrated pest management in arable cropping systems: analysis and reflections based on a European network. Eur. J. Agron. 83, 86–99.

Lemaire, G., Franzluebbers, A., de Carvalho, P.C.F., Dedieu, B., 2014. Integrated crop–livestock systems: strategies to achieve synergy between agricultural production and environmental quality. Agric. Ecosyst. Environ. 190, 4–8.

Moraes, A., Carvalho, P.C.F., Lustosa, S.B.C., Lang, C.R., Deiss, L., 2014. Research on integrated crop-livestock systems in Brazil. Rev. Ciênc. Agron. 45, 1024–1031.

Robertson, G.P., Swinton, S.M., 2005. Reconciling agricultural productivity and environmental integrity: a grand challenge for agriculture. Front. Ecol. Environ. 3, 38–46.